National Toxicology Program
Toxicity Report Series
Number 86

NTP Technical Report on the Toxicity Studies of

Cedarwood Oil (Virginia)

(CAS No. 8000-27-9)

Administered Dermally to F344/N Rats and B6C3F1/N Mice

November 2016

National Institutes of Health
Public Health Service
U.S. Department of Health and Human Services

FOREWORD

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Administered Dermally to F344/N Rats and B6C3F1/N Mice

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November 2016

National Institutes of Health
Public Health Service
U.S. Department of Health and Human Services

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PEER REVIEW

The draft report on the toxicity studies of cedarwood oil was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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CONTENTS

SUMMARY

Background

Virginia cedarwood oil is a widely used fragrance ingredient in many personal and household products such as soaps, cleaning products, and insecticides. We conducted short-term (3-month) tests to determine if there were any toxic effects of cedarwood oil on rats or mice. Because skin contact is common in humans, a dermal route of exposure was selected for these studies.

Methods

We applied solutions of cedarwood oil either neat or in ethanol to the skin on the backs of male and female rats and mice 5 days per week for 3 months. There were 10 rodents in each dose group. Doses were 6.25%, 12.5%, 25%, and 50% cedarwood oil in ethanol and 100% (neat) cedarwood oil. These doses corresponded to approximately 31.25 to 500 milligrams (mg) of cedarwood oil per kilogram (kg) of body weight in rats and 125 to 2,000 mg/kg in mice. One control group (vehicle control) received ethanol alone and served as the control group for dose groups that were dosed with 6.25% to 50% cedarwood oil in ethanol. Another control group (untreated control) received no administration of ethanol and served as the control group for the dose group exposed to 100% cedarwood oil. During the course of the studies, samples were collected for estrous cycle characterization. At the end of the studies, samples were collected for hematology, reproductive tissue evaluations, and genetic toxicology studies (mice only), and more than 40 tissues were collected from each animal for histopathology diagnosis.

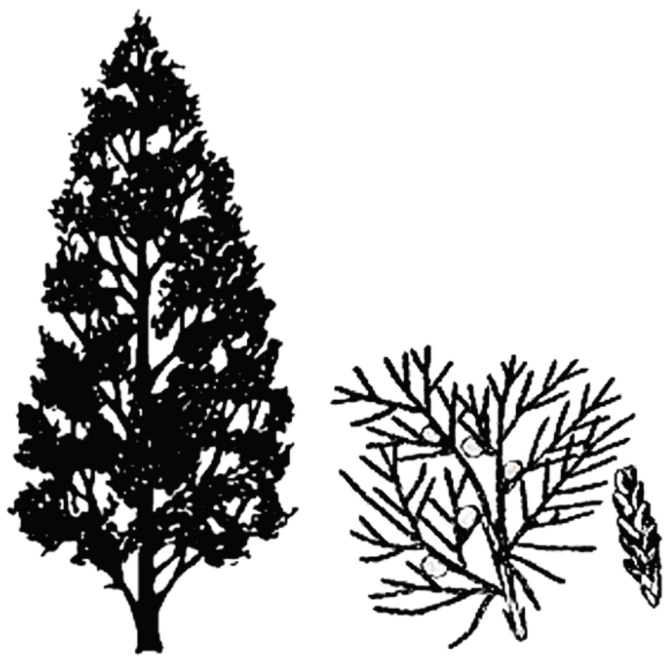
Results

Survival was decreased in male and female mice dosed with 100% cedarwood oil. Mean body weights of animals exposed to cedarwood oil were decreased in the 50% and 100% groups of male and female rats and in the 25% and 50% groups of male and female mice. At the site of cedarwood oil application on the skin, there were several clinical observations (irritation, thicker skin, and ulcers) that corresponded with the occurrence of skin lesions (hyperplasia, hyperkeratosis, inflammation, and fibrosis). These skin lesions occurred in rats dosed with 12.5% to 100% cedarwood oil, in all dosed groups of mice, and were more severe in mice.

Conclusions

We conclude that dermal exposure to cedarwood oil for 3 months resulted in skin lesions at the site of cedarwood oil application in both rats and mice.

ABSTRACT



VIRGINIA CEDARWOOD OIL

CAS No. 8000-27-9

Synonyms: Cedar oil; cedarwood oil; oil of cedarwood; red cedarwood oil

Virginia cedarwood oil (hereafter referred to as cedarwood oil) is extracted from *Juniperus virginiana* trees by steam distillation and contains cedrol, cedrene, cedrenol, cedral, cuperene, thujopsene, and widdrol as primary components. Cedarwood oil is used as a fragrance in cosmetic products, as a pesticide, and as a source material for production of other fragrance materials with cedarwood odors. Cedarwood oil was nominated for toxicity testing by the National Cancer Institute based on widespread and potentially increasing human exposure to the substance, and a lack of toxicology data. The dermal route of administration was selected for these studies because it is the most common route of exposure in humans due to its frequent use as a pesticide and as a fragrance in household products and cosmetics. Male and female F344/N rats and B6C3F1/N mice were administered cedarwood oil dermally for 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

Groups of 10 male and 10 female rats and mice received no treatment (untreated control) or were administered cedarwood oil in 95% aqueous ethanol dermally at concentrations of 0% (vehicle control), 6.25%, 12.5%, 25%,

50%, or 100% (neat) 5 days per week for 14 weeks. Formulations were administered at a volume of 0.5 mL/kg body weight (rats) or 2.0 mL/kg (mice), which resulted in the mice receiving higher doses of cedarwood oil than rats in respect to the equivalent dose in mg/kg. Dose ranges for mice and rats were estimated as 125 to 2,000 mg cedarwood oil/kg body weight and 31.25 to 500 mg/kg, respectively. Statistical analyses of effects in dosed groups (except 100%) were conducted with comparisons to the appropriate vehicle control group; groups dosed with 100% cedarwood oil were compared to the appropriate untreated control group.

With the exceptions of two males in the 100% group, all rats survived to the end of the study. Final mean body weights and body weight gains of 50% and 100% males and females were significantly less than those of their respective control groups, and the mean body weight gain of male rats in the 25% group was significantly less than that of the vehicle control group. Treatment-related clinical observations included irritation, thickening, and ulceration of the skin at the site of application in most males in the 25% or greater groups and most females in the 12.5% or greater groups.

In rats, relative liver weights of males in the 50% and 100% groups, and absolute and relative liver weights of females in the 100% group were significantly greater than those in the respective control groups. The absolute kidney weight of 100% males and relative kidney weights of males in the 50% and 100% groups were significantly greater than those in the respective control groups. The absolute thymus weights of 25% and 100% males and the absolute and relative thymus weights of 100% females were significantly less than those in the respective control groups.

Compared to those in the respective control groups, the incidences of several nonneoplastic lesions of the skin at the site of application were significantly increased in dosed groups of male and female rats. The incidences of epidermal hyperplasia were significantly increased in 12.5% or greater males and all dosed groups of females. The incidences of epidermal hyperkeratosis and sebaceous gland hyperplasia were significantly increased in 25% or greater males and 12.5% or greater females. The incidences of epidermal ulcer were significantly increased in the 50% and 100% groups of males and females. The incidences of chronic active inflammation were significantly increased in 12.5% or greater males and females. The incidences of dermal fibrosis and hair follicle hyperplasia were significantly increased in 25% or greater males and females. Hematology effects included increased leukon (white blood cell and differential counts) in 100% males and 50% or greater females and treatment-related decreases in erythron (hematocrit, hemoglobin, and erythrocyte counts) in 100% males and females. In the kidney of rats, the incidences of renal tubule degeneration were significantly increased in 50% and 100% males compared to those in the respective control groups. In addition, the incidences of renal tubule granular casts and hyaline droplet accumulation were significantly increased in males in the 25% or greater groups compared to those in the respective control groups; the severity of these lesions increased with increasing dose. In the bone marrow of rats, the incidences of hyperplasia were significantly increased in 100% males and females compared to those in the untreated control groups.

Due to the severities of skin lesions at the site of application, all male and female mice in the 100% groups, one male and one female each in the 50% groups, and one male in the 12.5% group were euthanized during weeks 10, 11, and 12, respectively; all other male and female mice survived to the end of the study. The final mean body weights of 25% and 50% males and 12.5% or greater females and the mean body weight gains of 12.5% or greater males and females were significantly less than those of the vehicle control groups. Test article-related clinical observations included irritation, thickening, and ulceration of the skin at the site of application in most dosed mice.

In mice, the absolute liver weights of 50% males and females and relative liver weights of all dosed groups of males and females were significantly greater than those of the vehicle control groups. The absolute kidney weight of 50% females and relative kidney weights of 12.5% or greater females were significantly greater than those of the vehicle control groups. Absolute thymus weights of 25% and 50% males and 12.5% or greater females were significantly less than those of the vehicle controls.

The incidences of epidermal hyperplasia, hyperkeratosis, and ulcer and chronic active inflammation, dermal fibrosis, hair follicle hyperplasia, and sebaceous gland hyperplasia were significantly increased in 12.5% or greater groups of male and female mice compared to those in the respective control groups. In addition, compared to the occurrences in the vehicle control groups, the incidences of epidermal hyperplasia and hyperkeratosis and chronic active inflammation, hair follicle hyperplasia, and sebaceous gland hyperplasia were significantly increased in 6.25% males, and the incidences of epidermal hyperplasia, chronic active inflammation, and sebaceous gland hyperplasia were significantly increased in 6.25% females. Hematology effects included increased leukon (white blood cells and differential counts) in 25% or greater females and treatment-related decreases in erythron (hematocrit, hemoglobin, and erythrocyte counts) in 12.5% or greater males and 25% and 50% females. In the liver, the incidences of hepatocyte glycogen depletion were significantly increased in males and females in the 12.5% or greater groups compared to those in the respective control groups. In the thymus, the incidences of atrophy were significantly increased in 25% or greater males compared to those in the respective controls. In the kidney, the incidence of nephropathy was significantly increased in 100% males compared to that in the untreated controls.

Cedarwood oil was not mutagenic in *S. typhimurium* strains TA98, TA100, or TA102 with or without exogenous metabolic activation. No increase in micronucleated erythrocytes was seen in blood samples obtained from male B6C3F1/N mice treated with cedarwood oil for 3 months via dermal application; a small increase in micronucleated erythrocytes, judged to be equivocal, was seen in female B6C3F1/N mice, however. No significant alterations in the percentage of polychromatic erythrocytes (reticulocytes) were seen in male or female mice, suggesting that dermally-applied cedarwood oil did not induce bone marrow toxicity.

Under the conditions of the 3-month dermal studies with Virginia cedarwood oil, there were treatment-related lesions in male and female rats and mice. Skin (at the site of application) and kidney were the major targets from administration of cedarwood oil in both rats and mice. Additionally, the liver and the thymus were considered secondary targets of cedarwood oil administration as a result of the skin effects at the site of application in both rats and mice. The most sensitive measures of cedarwood oil administration in each species and sex were: increased incidences of skin (site of application) lesions in male [lowest-observed-effect-level (LOEL) = 12.5%; approximately equivalent to 62.5 mg/kg] and female (LOEL = 6.25%; approximately equivalent to 31.25 mg/kg) rats and increased incidences of skin (site of application) lesions (LOEL = 6.25%; approximately equivalent to 124 mg/kg) in male and female mice.

Summary of Findings Considered to be Toxicologically Relevant in Rats and Mice Administered Cedarwood Oil Dermally for 3 Months[a]

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations by dermal application	0% (UC), 0% (VC), 6.25%, 12.5%, 25%, 50%, or 100%	0% (UC), 0% (VC), 6.25%, 12.5%, 25%, 50%, or 100%	0% (UC), 0% (VC), 6.25%, 12.5%, 25%, 50%, or 100%	0% (UC), 0% (VC), 6.25%, 12.5%, 25%, 50%, or 100%
Survival rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10, 8/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 9/10, 10/10, 9/10, 0/10[c]	10/10, 10/10, 10/10, 10/10, 10/10, 9/10, 0/10[c]
Body weights	50% group 9% less than the vehicle control group; 100% group 14% less than the untreated control group	50% group 8% less than the vehicle control group; 100% group 11% less than the untreated control group	25% group 10% less than the vehicle control group; 50% group 14% less than the vehicle control group	12.5% group 12% less than the vehicle control group; 25% group 19% less than the vehicle control group; 50% group 13% less than the vehicle control group
Clinical observations	Irritation, thickening, and ulceration of the skin at the site of application	Irritation, thickening, and ulceration of the skin at the site of application	Irritation, thickening, and ulceration of the skin at the site of application	Irritation, thickening, and ulceration of the skin at the site of application
Organ weights	↑ Liver (relative[b]) ↑ Kidney (absolute and relative) ↓ Thymus (absolute)	↑ Liver (absolute and relative) ↓ Thymus (absolute and relative)	↑ Liver (absolute and relative) ↓ Thymus (absolute)	↑ Liver (absolute and relative) ↑ Kidney (absolute and relative) ↓ Thymus (absolute)
Hematology	↑ Total white blood cells ↑ Neutrophil counts	↑ Total white blood cells ↑ Neutrophil counts	↑ Neutrophil counts ↓ Hematocrit ↓ Hemoglobin ↓ Erythrocytes	↑ Total white blood cells ↑ Neutrophil counts ↓ Hematocrit ↓ Hemoglobin ↓ Erythrocytes
Reproductive toxicity	None	None	None	None
Nonneoplastic effects	<u>Skin (site of application)</u>: epidermis, hyperplasia (0/10, 0/10, 2/10, 4/10, 10/10, 10/10, 9/10); epidermis, hyperkeratosis (0/10, 0/10, 0/10, 1/10, 7/10, 10/10, 10/10); sebaceous gland, hyperplasia (0/10, 0/10, 0/10, 0/10, 9/10, 10/10, 10/10); epidermis, ulcer (0/10, 0/10, 0/10, 1/10, 1/10, 7/10, 8/10); inflammation, chronic active (0/10, 0/10, 1/10, 4/10, 9/10, 10/10, 10/10); dermis, fibrosis (0/10, 0/10, 0/10, 0/10, 7/10, 10/10, 10/10); hair follicle, hyperplasia (0/10, 0/10, 0/10, 0/10, 9/10, 10/10, 9/10)	<u>Skin (site of application)</u>: epidermis, hyperplasia (0/10, 0/10, 4/10, 7/10, 10/10, 10/10, 10/10); epidermis, hyperkeratosis (0/10, 0/10, 1/10, 5/10, 10/10, 10/10, 10/10); sebaceous gland, hyperplasia (0/10, 0/10, 0/10, 4/10, 9/10, 10/10, 10/10); epidermis, ulcer (0/10, 0/10, 0/10, 1/10, 1/10, 4/10, 10/10); inflammation, chronic active (0/10, 0/10, 0/10, 7/10, 10/10, 10/10, 10/10); dermis, fibrosis (0/10, 0/10, 0/10, 1/10, 7/10, 8/10, 10/10); hair follicle, hyperplasia (0/10, 0/10, 0/10, 1/10, 9/10, 10/10, 10/10)	<u>Skin (site of application)</u>: epidermis, hyperplasia (0/10, 0/9, 10/10, 10/10, 10/10, 10/10, 10/10); epidermis, hyperkeratosis (0/10, 0/9, 7/10, 10/10, 10/10, 10/10, 10/10); epidermis, ulcer (0/10, 0/9, 2/10, 8/10, 10/10, 9/10, 10/10); inflammation, chronic active (0/10, 0/9, 10/10, 10/10, 10/10, 10/10, 10/10); dermis, fibrosis (0/10, 0/9, 3/10, 10/10, 10/10, 10/10, 10/10); hair follicle, hyperplasia (0/10, 0/9, 10/10, 10/10, 10/10, 10/10, 10/10); sebaceous gland, hyperplasia (0/10, 0/9, 10/10, 10/10, 10/10, 10/10, 10/10)	<u>Skin (site of application)</u>: epidermis, hyperplasia (0/10, 0/10, 10/10, 10/10, 10/10, 10/10, 10/10); epidermis, hyperkeratosis (0/10, 0/10, 2/10, 10/10, 10/10, 10/10, 10/10); epidermis, ulcer (0/10, 0/10, 2/10, 6/10, 9/10, 10/10, 10/10); inflammation, chronic active (0/10, 0/10, 10/10, 10/10, 10/10, 10/10, 10/10); dermis, fibrosis (0/10, 0/10, 2/10, 10/10, 10/10, 10/10, 10/10); hair follicle, hyperplasia (0/10, 0/10, 3/10, 10/10, 10/10, 10/10, 10/10); sebaceous gland, hyperplasia (1/10, 0/10, 4/10, 10/10, 10/10, 10/10, 10/10)

Summary of Findings Considered to be Toxicologically Relevant in Rats and Mice Administered Cedarwood Oil Dermally for 3 Months

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Nonneoplastic effects (continued)	Kidney: renal tubule, degeneration (2/10, 4/10, 5/10, 6/10, 7/10, 10/10, 10/10); renal tubule, casts granular (0/10, 0/10, 0/10, 0/10, 5/10, 10/10, 10/10); accumulation, hyaline droplet (0/10, 0/10, 0/10, 0/10, 10/10, 10/10, 10/10)		Kidney: nephropathy (0/10, 2/10, 0/10, 1/10, 2/10, 3/10, 5/10)	
Genetic toxicology				
Bacterial gene mutations:		Negative in *S. typhimurium* strains TA98, TA100, and TA102 with and without S9		
Micronucleated erythrocytes Mouse peripheral blood *in vivo*:		Negative in males and equivocal in females		

[a] Reported treatment-related effects are in comparison to concurrent controls; untreated control (UC) compared to 100% group and ethanol vehicle control (VC) compared to all other dosed groups

[b] Relative to body weight

[c] Due to the severity of skin lesions, all 100% mice were euthanized during week 10

INTRODUCTION

VIRGINIA CEDARWOOD OIL

CAS No. 8000-27-9

Synonyms: Cedar oil; cedarwood oil; oil of cedarwood; red cedarwood oil

CHEMICAL AND PHYSICAL PROPERTIES

Cedarwood oils can be extracted from several members of the Cupressaceae family, which encompasses true cedars, junipers, and cypresses. The three most prevalent cedarwood oil products in the United States are Virginia cedarwood oil (*Juniperus virginiana*), Texas cedarwood oil (*Juniperus mexicana* or *Juniperus ashei*), and Western red cedar (*Thuja plicata*) (*Merck*, 1989). Western red cedar is the least commonly used of the three (Coppen, 1995; Ciesla, 1998).

The most common components of cedarwood oils are sesquiterpene hydrocarbons and include cedrol, α-cedrene, cedrenol, cedral, cuparene, thujopsene, and widdrol, though the relative percentages can differ depending on the origin of the cedar trees (Merory, 1968; *Ullmann's*, 1985; USEPA, 1993; Franz *et al*., 1998; Dunford *et al*., 2007). The International Organization for Standardization set the following minimum and maximum standards for the components that make up the chromatographic profile of cedarwood oil (from *Juniperus virginiana*): α-cedrene

(20% to 35%), β-cedrene (4% to 8%), thujopsene (10% to 25%), cuparene (1.5% to 7%), cedrol (16% to 25%), and widdrol (2% to 5%) (ISO, 2004).

Cedarwood oil is a colorless to pale brown viscous liquid with a cedarwood odor and bitter taste (Merory, 1968; *Ullmann's*, 1985). It is insoluble in water but soluble in ethanol or ether (HSDB, 2014) and may solidify at room temperature (*Ullmann's*, 1985).

Production, Use, and Human Exposure

Virginia cedarwood oil is extracted from *Juniperus virginiana* trees by steam distillation (*Ullmann's*, 1985). Cedarwood oil of unspecified origin is used commonly in fragrance formulations, and production for this purpose exceeds 100,000 pounds per year (RIFM, 1974; *Ullmann's*, 1985). Several cedarwood oil components can be isolated and acetylated for use in fragrances. Of the chemical components that make up cedarwood oil, cedrene, thujopsene, and cedrol are commonly isolated and often acetylated in the production of fragrance materials such as cedryl acetate and cedryl methylether (*Ullmann's*, 1985). These extracts can be found in many cosmetic products (i.e., perfumes, lotions) that may be applied to most parts of the body and may come into contact with the ocular and nasal mucosae. Frequent application of these products on a daily basis leads to long-term chronic exposure. The cosmetics industry no longer reports concentration of use values to the United States Food and Drug Administration (*Fed. Regist*., 1992). However, data submitted to the FDA indicated that the maximum use concentration ranges from the 1984 product formulation data for Juniper Extract and Juniper Tar in cosmetics were 0.1% to 1%, and 1% to 5%, respectively (CIR, 2001). Limited or no toxicity was found in rats and rabbits following dermal application of either Western juniper oil (0.5%, 5%, or 50%) or Port-Orford cedar extract (0.5%, 5%, or 50%) when tested using a local lymph node assay and an acute dermal irritation study (Craig *et al*., 2004).

As a pesticide, cedarwood oil is used as an insect repellent in sunscreens, pet collars, and for horses (USEPA, 2009). The potential use of cedarwood oil as a topical mosquito repellent was explored, but found to be ineffective against multiple mosquito species (Curtis *et al.*, 1987; Barnard, 1999). However, it was found to be effective with an average knock-down range of 20% to 80% when applied directly to mosquitoes, cockroaches, and houseflies (Singh *et al*., 1984). Virginia cedarwood oil was also found to be effective as a barrier repellent to red imported fire ants and black-legged tick nymphs (Eller *et al*., 2014). Additionally, it is used in blocks and as a liquid spray for repelling moths and mildew from clothing and fabrics (USEPA, 1993, 2009). *In vitro*, Japanese cedarwood oils have been found to have anti-fungal properties against *Trichophyton rubrum* through the inhibition of DNA polymerase (Takao *et al*., 2012).

Historically, in ancient Rome and in early America, cedarwood oil was taken orally to induce abortion (Acevedo, 1979; Gosselin *et al*., 1984; HSDB, 2014). Data on current human use of cedarwood oil as an abortifacient are minimal. However, exposure to the bark, leaves, and berries of western juniper trees (*Juniperus occidentalis*) has been shown to induce abortions in late-term cattle in Oregon (Welch *et al*., 2013).

Based on the 1981 to 1983 National Occupational Exposure Survey, cedarwood oil was used in 7,990 facilities, and 117,858 employees were exposed to cedarwood oil (NIOSH, 1990). From this NIOSH report, the scope of occupational facilities in which cedarwood oil is used is large and includes industrial settings, medical fields, and esthetics and beauty care.

REGULATORY STATUS

Cedarwood oil alcohols and terpenes are listed as synthetic flavoring substances and adjuvants that are permitted by the United States Food and Drug Administration (21 CFR, § 172.515) for direct addition to food for human consumption. Cedarwood oil was registered as a pesticide in the United States in 1960 (USEPA, 1993). Currently, cedarwood oil is listed as a minimum risk pesticide and exempted from the Federal Insecticide, Fungicide, and Rodenticide Act regulations (40 CFR, § 152.25). Cedarwood oil is listed in the Toxic Substances Control Act Inventory (USEPA, 2013). However, no limits on recommended or permissible exposures are available.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

There are very limited data in the literature on the disposition of cedarwood oil and its components. Following gavage administration of 25 (males only), 50 (females only), or 100 mg/kg α-cedrene to male and female Sprague Dawley rats, the chemical was absorbed slowly with a T_{max} between 2.8 and 4.4 hours (Hong *et al.*, 2013; Kim *et al.*, 2015). The values estimated for absolute bioavailability were 42% (males, 25 mg/kg), 49% (females, 50 mg/kg), 71% (female, 100 mg/kg), or 85% (male, 100 mg/kg). Tissue distribution was investigated only in female rats, and α-cedrene was extensively distributed to tissues with tissue:plasma ratios greater than 1 for all tissues with lipid showing the highest tissue:plasma ratio. Plasma elimination half-life was between 2 and 5.9 hours depending on the study (Hong *et al.*, 2013; Kim *et al.*, 2015). In females, C_{max} and AUC values increased proportionally to the dose (Kim *et al.*, 2015). There were no sex- or dose-related differences in the disposition of α-cedrene. The urinary excretion of unmetabolized α-cedrene following gavage administration was equal to or less than 0.017%.

TOXICITY

Experimental Animals

The oral LD_{50}value for cedarwood oil is greater than 5 g/kg body weight in rats, and the dermal LD_{50} in rabbits is also greater than 5g/kg (RIFM, 1974).

Humans

Virginia cedarwood oil (1% or 5%) in petrolatum was applied to 95 human patch test subjects and no skin irritation was seen on days 3 or 4 (Frosch *et al.*, 1995). Furthermore, several additional studies in humans have shown similar results with no skin irritation effects after Virginia cedarwood oil was topically applied to the skin over periods from 24 hours to 4 days in concentrations ranging from 0.2% to 20% (Kligman, 1966; Fujii *et al.*, 1972; RIFM, 1974;

Abifadel *et al*., 1992). Additional dermatologic assessments with acetyl cedrene, a common constituent of cedarwood oil, also demonstrated limited human toxicity in multiple irritation tests at concentrations up to 30% (Belsito *et al.,* 2013).

Reproductive and Developmental Toxicity

Oral exposure of pregnant Sprague Dawley rats to the cedarwood oil derivative acetyl cedrene at concentrations of 25, 50, or 100 mg/kg per day on gestation days 7 to 17 resulted in no significant fetal effects and minor maternal effects such as decreased body weight gains and feed consumption; the no-observed-adverse-effect levels were 50 and 100 mg/kg per day for the dams and fetuses, respectively (Lapczynski *et al*., 2006). In a separate study, β-thujaplicin (another cedarwood oil derivative) was orally administered to pregnant ICR mice on gestation day 9; evaluation of the fetuses on gestation day 18 found that the chemical induced several malformations at doses of 560 mg/kg or greater, including cleft lip, cleft palate, and facial dysmorphism (Ogata *et al*., 1999).

Carcinogenicity

No information on the carcinogenicity of cedarwood oil in experimental animals or humans was found in a review of the literature.

Genetic Toxicity

No information on the genotoxicity of cedarwood oil was found in the literature.

Study Rationale

Cedarwood oil was nominated for study by the National Cancer Institute based on widespread and increasing human exposure to the substance and a lack of toxicology data. Due to the frequent use of cedarwood oil as a fragrance material and a pesticide, the dermal route of administration was selected for these studies because it is the most common route of exposure in humans.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Virginia Cedarwood Oil

Prior to selecting the test article, the NTP investigated the composition of selected components in six lots that were procured as Virginia cedarwood oil from three suppliers. The chromatographic profiles of the six lots were similar to each other. The composition of α-cedrene, β-cedrene, thujopsene, cuparene, and cedrol in these lots ranged from 18% to 40%, 4% to 8%, 16% to 31%, 2% to 4%, and 19% to 26%, respectively. Due to the close similarities between the procured lots, the lot selected for testing was based upon the availability in bulk quantity.

For the current 3-month dermal studies, Virginia cedarwood oil was obtained from Texarome, Inc. (Leakey, TX), in one lot (T122303DP). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO) and the study laboratory at Battelle Columbus Operations (Columbus, OH) (Appendix F). Reports on analyses performed in support of the cedarwood oil studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a pale yellow oily liquid with a cedar odor, was identified as cedarwood oil using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy.

Physical properties for lot T122303DP were determined by the analytical chemistry laboratory; optical rotation was –26.1° and specific gravity was 0.9581 at 24.8° C, refractive index was 1.5053 at 19° C, and viscosity was 22.6 cP at 22.2° C. The determined values for optical rotation, specific gravity, and refractive index were consistent with the reported values for cedarwood oil (Adams, 1991).

The purity of lot T122303DP was determined based on a chromatography profile of the major components obtained by using gas chromatography (GC) with flame ionization detection (FID). Five compounds (α-cedrene, β-cedrene, thujopsene, cuparene, and cedrol) were selected as marker compounds and quantitated using standards obtained from Sigma-Aldrich Corporation (St. Louis, MO); the percentages were 27.01% α-cedrene, 6.05% β-cedrene, 19.64% thujopsene, 0.96% cuparene, and 21.83% cedrol. The components of lot T122303DP were also determined using GC with mass spectrometry (MS) detection. Ten marker compounds having peak areas of at least 1% of the total peak area were identified in this analysis: α-cedrene, β-cedrene, thujopsene, β-chamigrene, α-alaskene, α-cuprenene, β-himachalene, cuparene, cedrol, and widdrol. Headspace analyses for volatiles were conducted on samples of this lot using GC/MS both before and after homogenization at 50° C, and 15 components were identified in the chromatographic profiles; limonene (or an isomer), iso-italicene (or an isomer), italicene (or an isomer), α-cedrene, *cis*-β-farnesene (or an isomer), β-selinene (or an isomer), β-cedrene, thujopsene, β-chamigrene (or an isomer), α-alaskene (or an isomer), α-cuprenene (or an isomer), β-himachalene (or an isomer), cuparene, cedrol, and widdrol. No significant differences were observed between samples analyzed before and after homogenization. Taken together, the results of these characterization assays indicated that the composition of lot T122303DP was consistent with that reported in the literature for cedarwood oil (Adams, 1989, 1991, 2001).

Stability studies of the bulk chemical were performed for samples stored at –20°, 5°, 25°, or 60° C in sealed amber glass vials; additional freeze/thaw analyses were also performed every 2 to 3 days during the 2-week stability studies. Stability was confirmed for at least 2 weeks for samples stored at temperatures up to 25° C in sealed amber glass vials. Freeze/thaw analyses indicated no decomposition due to repeated freezing and thawing.

To ensure stability, the bulk chemical was stored at approximately 25° C in the original sealed amber glass shipping bottles. Periodic reanalyses of the bulk chemical were performed during the 3-month studies using GC/FID and no degradation of the bulk chemical was detected.

Ethanol

USP grade 95% ethanol was obtained from Spectrum Chemicals & Laboratory Products (Gardena, CA) in one lot (TP0179) and from AAPER Alcohol (Shelbyville, KY) in one lot (02K2JWB). Lot TP0179 was used in the 3-month dermal studies, and lot 02K2JWB was used in the dose formulation stability studies.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared four times by mixing cedarwood oil and 95% ethanol to give the required concentrations (Table F2). The dose formulations were stored at approximately 25° C in amber glass bottles sealed with Teflon®-lined lids for up to 34 days.

Homogeneity and stability studies of a 9.5 mg/mL (0.95%) formulation in 95% ethanol were performed by the analytical chemistry laboratory using GC/FID. Based on these studies, homogeneity was confirmed and stability was confirmed for cedarwood oil formulations stored in amber glass containers sealed with Teflon®-lined lids for 1 day with expected losses of 6.2% or less for all components or stored for 42 days with expected losses of 10.4% or less. Cedarwood oil formulations were stable for up to 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of cedarwood oil were conducted by the study laboratory using GC/FID. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations analyzed for rats and all 14 dose formulations for mice were within 10% of the target concentrations (Table F3). Animal room samples of these dose formulations were also analyzed; all 15 for rats and all 14 for mice were within 10% of the target concentrations.

ANIMAL SOURCE

Male and female F344/N rats and B6C3F1/N mice were obtained from the NTP colony at Taconic Farms, Inc. (Germantown, NY).

ANIMAL WELFARE

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

3-MONTH STUDIES

On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 13 or 14 days (rats) or 11 or 12 days (mice); rats were 6 to 7 weeks old and mice were 5 to 6 weeks old on the first day of dosing at the start of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix H). All test results were negative.

Groups of 10 male and 10 female rats and mice received no treatment (untreated control) or were administered cedarwood oil in 95% aqueous ethanol dermally at concentrations of 0% (vehicle control), 6.25%, 12.5%, 25%, 50%, or 100% (neat) 5 days per week for 14 weeks. Formulations were administered at a volume of 0.5 mL/kg body weight (rats) or 2.0 mL/kg (mice). These corresponded to applied doses of 31.25 to 500 mg cedarwood oil/kg body weight in rats and 125 to 2,000 mg/kg in mice. Doses were applied using a repeating pipette with a disposable tip to a consistent area that was shaved weekly. The shaved area was on the dorsal surface just posterior to the scapulae to the base of the tail and larger than the application site. Feed and water were available *ad libitum*. Rats and mice were housed individually. All animals were weighed and clinical observations were recorded initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1. The feed was acceptable for use and information on feed composition and contaminants is provided in Appendix G.

Animals were anesthetized with a carbon dioxide/oxygen mixture, and blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice at the end of the 3-month studies for hematology analyses. Blood samples were collected in tubes containing EDTA as the anticoagulant. Parameters were measured using an ADVIA® 120 Hematology Analyzer (Bayer, Inc., Tarrytown, NY) using reagents supplied by the manufacturer. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility or vaginal cytology evaluations on male and female rats in the vehicle control, 12.5%, 25%, and 50% groups, and male and female mice in the vehicle control, 6.25%, 12.5%, and 25% groups. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal euthanasia, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrone's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Additional sections of the kidney from male rats were stained with the Mallory-Heidenhain stain for protein. Complete histopathologic examinations were performed by the study laboratory pathologist on rats in the untreated control, vehicle control, and 100% groups and mice in the untreated control, vehicle control, 50%, and 100% groups; the adrenal cortex (males), bone marrow, kidney (males only), liver, skin at the site of application, and thyroid gland of rats and the bone marrow, kidney (males only), liver, lymph nodes, skin at the site of application, spleen, and thymus of mice were examined in all remaining dosed groups. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s) and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

TABLE 1
Experimental Design and Materials and Methods in the 3-Month Dermal Studies of Cedarwood Oil

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

Strain and Species
F344/N rats
B6C3F1/N mice

Animal Source
Taconic Farms, Inc. (Germantown, NY)

Time Held Before Studies
Rats: 13 (males) or 14 (females) days
Mice: 11 (females) or 12 (males) days

Average Age When Studies Began
Rats: 6 to 7 weeks
Mice: 5 to 6 weeks

Date of First Dose
Rats: June 15 (males) or 16 (females), 2005
Mice: June 13 (females) or 14 (males), 2005

Duration of Dosing
5 days per week for 14 weeks

Date of Last Dose
Rats: September 14 (males) or 15 (females), 2005
Mice: September 12 (females) or 13 (males), 2005

Necropsy Dates
Rats: September 15 (males) or 16 (females), 2005
Mice: September 13 (females) or 14 (males), 2005

Average Age at Necropsy
19 to 20 weeks

Size of Study Groups
10 males and 10 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
1

Method of Animal Identification
Tail tattoo

Diet
Irradiated NTP-2000 open formula wafer feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum,* changed at least weekly

Water
Tap water (City of Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*

Cages
Polycarbonate [Allentown Caging Equipment Company, Allentown, NJ (rats), or Lab Products, Inc., Seaford, DE (mice)], changed weekly

TABLE 1
Experimental Design and Materials and Methods in the 3-Month Dermal Studies of Cedarwood Oil

Bedding
Heat-treated, irradiated hardwood Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly

Rack Filters
Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH), changed every 2 weeks

Racks
Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 10/hour

Concentrations
Untreated control, 0% (vehicle control), 6.25%, 12.5%, 25%, 50%, or 100% (neat)

Type and Frequency of Observation
Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly thereafter, and at the end of the studies.

Method of Euthanasia
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.

Clinical Pathology
Blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice at the end of the studies for hematology.
Hematology: hematocrit; hemoglobin; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials

Histopathology
Complete histopathology was performed on rats in the untreated control, vehicle control, and 100% groups and mice in the untreated control, vehicle control, 50%, and 100% groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with mainstem bronchus), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin (site of application), spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. The adrenal cortex (males), bone marrow, kidney (males), liver, skin at the site of application, and thyroid gland of rats and the bone marrow, kidney (males), liver, lymph nodes, skin at the site of application, spleen, and thymus of mice were examined in the remaining dosed groups.

Sperm Motility and Vaginal Cytology
At the end of the studies, sperm samples were collected from male rats in the vehicle control, 12.5%, 25%, and 50% groups and male mice in the vehicle control, 6.25%, 12.5%, and 25% groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats in the vehicle control, 12.5%, 25%, and 50% groups and female mice in the vehicle control, 6.25%, 12.5%, and 25% groups for vaginal cytology evaluations.

STATISTICAL METHODS

Responses at concentrations up to 50% were compared to those of the vehicle control group; responses at 100% were compared to those of the untreated control group. In addition, vehicle control groups were compared to untreated control groups for selected rat data.

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test (Gart *et al*., 1979), a procedure based on the overall proportion of affected animals, was used to determine significance between dosed and untreated control or vehicle control animals.

Analysis of Continuous Variables

For dosed groups compared to the vehicle control groups, two approaches were employed to assess the significance of pairwise comparisons in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the vehicle control group using the Fisher exact test (Gart *et al*., 1979). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the vehicle control group and each dosed group was tested using chi-square statistics.

For comparison of the 100% (neat) groups to the untreated control groups, a *t*-test was used to determine significant differences in organ and body weight data for rats, and a Wilcoxon's (1945) rank sum test was used for hematology data for rats.

Quality Assurance Methods

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 3-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity Study Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Toxicity Study Report.

Genetic Toxicology

Salmonella typhimurium Mutagenicity Test Protocol

Testing procedures used for cedarwood oil followed protocols reported by Zeiger *et al*. (1992). Cedarwood oil was sent to the laboratory as a coded sample. It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, and TA102 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rats) for 20 minutes at 37º C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37º C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of cedarwood oil. The high dose was limited by toxicity to 33 µg/plate without S9 mix and 333 µg/plate with S9.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al*. (1990). At the termination of the 3-month study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronucleated cells in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 and the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. Trials with either a significant trend or a significant dose are judged to be equivocal. The absence of a trend and a significant dose results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight-of-evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Report represent a scientific judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

3-MONTH STUDY IN RATS

With the exceptions of two males in the 100% group, which were removed due to severe skin lesions, all rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of 50% and 100% males and females were significantly less than those of their respective control groups and the mean body weight gain of male rats in the 25% group was significantly less than that of the vehicle control group (Table 2 and Figure 1). Test article-related clinical observations included irritation, thickening, and ulceration of the skin at the site of application in most males in the 25% or greater groups and most females in the 12.5% or greater groups.

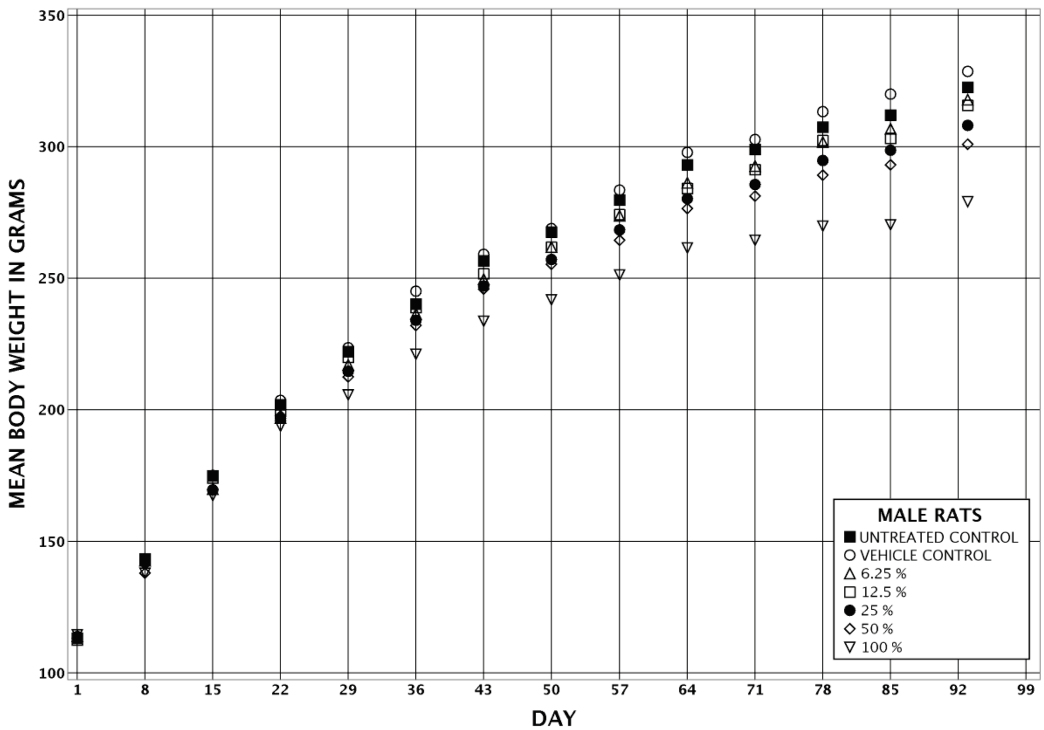
The hematology data for rats are presented in Table B1. There was a small (30% to 40%) increase in the total white blood cell counts of 50% females and the 100% groups of both sexes. The increased leukon was characterized most consistently by two-to three-fold increases in neutrophil counts, and to a lesser extent, increases in monocyte counts in the 100% male and female groups. Neutrophil counts were also minimally increased (30% to 40%) in the 50% male and female groups. A minimal decrease in erythron (5%) was evidenced by small decreases in the hematocrit value and hemoglobin concentration of 100% females and the erythrocyte count of 100% males.

There were no changes in the number of sperm or spermatids, sperm motility, or testis and epididymis weights of dosed males (Table D1). There were no estrous cycle changes in dosed females (Tables D2 and D3; Figure D1). Under the conditions of this study, cedarwood oil applied dermally did not exhibit the potential to be a reproductive toxicant in male or female F344/N rats.

TABLE 2
Survival and Body Weights of Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

Concentration (%)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls[c] (%)
Male					
Untreated Control	10/10	113 ± 2	323 ± 4	209 ± 3	
Vehicle Control	10/10	114 ± 2	329 ± 4	215 ± 4	
6.25	10/10	114 ± 2	318 ± 5	204 ± 4	97
12.5	10/10	113 ± 2	316 ± 6	203 ± 5	96
25	10/10	114 ± 2	308 ± 10	195 ± 8*	94
50	10/10	113 ± 2	301 ± 8*	188 ± 8**	92
100	8/10[d]	115 ± 2	279 ± 7††	166 ± 7††	87
Female					
Untreated Control	10/10	102 ± 1	200 ± 4	98 ± 4	
Vehicle Control	10/10	103 ± 2	197 ± 2	94 ± 2	
6.25	10/10	102 ± 2	199 ± 4	97 ± 3	101
12.5	10/10	103 ± 1	194 ± 5	91 ± 4	98
25	10/10	102 ± 1	193 ± 4	91 ± 3	98
50	10/10	102 ± 1	182 ± 4*	80 ± 3**	92
100	10/10	102 ± 2	179 ± 3††	78 ± 3††	90

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
†† Significantly different ($P \leq 0.01$) from the untreated control group by a *t*-test
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Dosed groups are compared to the vehicle control groups, except 100% groups are compared to the untreated control groups.
[d] Week of deaths: 12



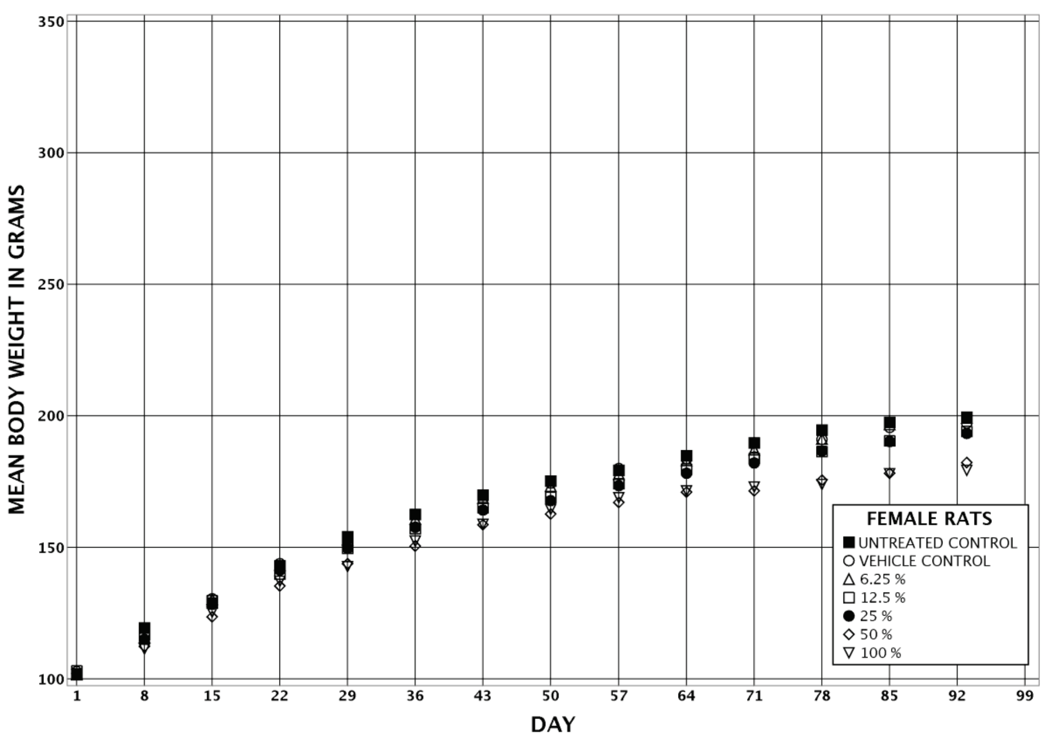


FIGURE 1
Growth Curves for Rats Administered Cedarwood Oil Dermally for 3 Months

Relative liver weights of males in the 50% and 100% groups, and absolute and relative liver weights of females in the 100% group were significantly greater than those in the respective control groups (Tables 3 and C1). The absolute kidney weight of 100% males and relative kidney weights of males in the 50% and 100% groups and females in the 25% or greater groups were significantly greater than those in the respective control groups. However, the kidney weight changes in females are most likely attributable to overall body weight changes. The absolute thymus weights of 25% and 100% males and the absolute and relative thymus weights of 100% females were significantly less than those in the respective control groups. The organ weight changes in the liver, kidney, and thymus were not accompanied by any significant histopathologic lesions.

TABLE 3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Male							
n	10	10	10	10	10	10	8
Necropsy body wt	323 ± 4	329 ± 4	318 ± 5	316 ± 6	308 ± 10	301 ± 8*	279 ± 7††
R. Kidney							
Absolute	1.09 ± 0.02	1.15 ± 0.02†	1.08 ± 0.03	1.10 ± 0.02	1.12 ± 0.04	1.19 ± 0.03	1.33 ± 0.05††
Relative	3.37 ± 0.05	3.51 ± 0.04†	3.40 ± 0.04	3.50 ± 0.07	3.63 ± 0.06	3.96 ± 0.08**	4.77 ± 0.11††
Liver							
Absolute	12.42 ± 0.37	12.75 ± 0.26	12.91 ± 0.38	12.47 ± 0.31	12.60 ± 0.49	13.07 ± 0.37	13.43 ± 0.42
Relative	38.55 ± 1.21	38.79 ± 0.58	40.58 ± 0.97	39.52 ± 0.75	40.89 ± 0.86	43.49 ± 0.84**	48.18 ± 1.31††
Thymus							
Absolute	0.282 ± 0.013	0.282 ± 0.006	0.277 ± 0.008	0.280 ± 0.010	0.245 ± 0.011*	0.271 ± 0.014	0.223 ± 0.016†
Relative	0.875 ± 0.044	0.857 ± 0.014	0.870 ± 0.023	0.887 ± 0.033	0.798 ± 0.032	0.904 ± 0.049	0.797 ± 0.051
Female							
Necropsy body wt	200 ± 4	197 ± 2	199 ± 4	194 ± 5	193 ± 4	182 ± 4*	179 ± 3††
R. Kidney							
Absolute	0.72 ± 0.01	0.71 ± 0.02	0.73 ± 0.02	0.71 ± 0.02	0.74 ± 0.02	0.70 ± 0.02	0.75 ± 0.01
Relative	3.59 ± 0.07	3.59 ± 0.05	3.65 ± 0.04	3.63 ± 0.05	3.85 ± 0.06**	3.86 ± 0.07**	4.17 ± 0.09††
Liver							
Absolute	6.97 ± 0.18	7.02 ± 0.18	6.93 ± 0.23	7.10 ± 0.19	7.14 ± 0.18	6.80 ± 0.21	7.58 ± 0.13†
Relative	34.94 ± 0.60	35.62 ± 0.79	34.74 ± 0.55	36.60 ± 0.62	36.98 ± 0.56	37.28 ± 0.56	42.30 ± 0.42††
Thymus							
Absolute	0.279 ± 0.016	0.240 ± 0.006†	0.263 ± 0.012	0.245 ± 0.013	0.244 ± 0.008	0.225 ± 0.009	0.213 ± 0.009††
Relative	1.395 ± 0.071	1.220 ± 0.030†	1.320 ± 0.055	1.265 ± 0.064	1.262 ± 0.036	1.236 ± 0.046	1.189 ± 0.055††

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
† Significantly different ($P \leq 0.05$) from the untreated control group by *t*-test
†† $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Compared to those in the respective control groups, the incidences of several nonneoplastic lesions of the skin at the site of application were significantly increased in dosed groups of males and females (Tables 4, A1, and A2). The incidences of epidermal hyperplasia were significantly increased in 12.5% or greater males and all dosed groups of females. The incidences of epidermal hyperkeratosis and sebaceous gland hyperplasia were significantly increased in 25% or greater males and 12.5% or greater females. The incidences of epidermal ulcer were significantly increased in the 50% and 100% groups of males and females. The incidences of chronic active inflammation were significantly increased in 12.5% or greater males and females. The incidences of dermal fibrosis and hair follicle hyperplasia were significantly increased in 25% or greater males and females. Compared to normal skin (Plates 1 and 2), epidermal hyperplasia was characterized by increased thickness of the epidermis due to an increase in the number of epithelial cell layers (Plates 3 and 4). Hyperplasia frequently extended into and thickened the epithelium of the hair follicles and there were increased profiles of the hair follicle deep in the adjacent subcutaneous layers. Epithelial thicknesses of three, four, five, or greater than five layers of cells represented severity grades of minimal, mild, moderate, and marked, respectively. Epidermal hyperkeratosis generally accompanied hyperplasia and consisted of increased thickness of the epidermal keratin layer (Plates 5 and 6). There were both ortho- and parakeratotic hyperkeratoses and the severities were determined by the number of increased cell layers compared to the respective controls. Frequently, there were dense accumulations of degenerate neutrophils within the keratin layers. Epidermal ulcer was characterized by focal, complete loss of the epidermis, often associated with necrosis of the underlying superficial dermis and filling of the epidermal defect with degenerate and/or necrotic cellular debris and degenerate inflammatory cells (Plate 7). The severity was based on the size of the ulcers and the number of affected areas in the skin section. Chronic active inflammation was characterized by infiltrates of neutrophils and macrophages in varying numbers (increasing with dose) throughout the dermis with infrequent extension into the hypodermis (Plates 8 and 9). Occasionally, focal infiltrates of primarily neutrophils mixed with low numbers of macrophages were within the epidermis, hair follicles, and less frequently sebaceous glands. Dermal fibrosis was often associated with dermal inflammatory cell infiltrates and was characterized by increased amounts of collagenous/fibrous connective tissue in the dermis (Plate 10). Hair follicle hyperplasia was characterized by increased numbers of cross-sectional hair follicle profiles in association with thickening of the follicular epithelium that was an extension of the hyperplastic change that occurred in the epidermis (Plates 3 and 4). Sebaceous gland hyperplasia was characterized by an increase in the size of the sebaceous glands due to increased number and size of the alveolar cells (Plates 3 and 4).

TABLE 4
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Male							
Number Examined Microscopically	10	10	10	10	10	10	10
Epidermis, Hyperplasia[a]	0	0	2 (1.0)[b]	4* (1.3)	10** (2.0)	10** (2.9)	9†† (2.9)
Epidermis, Hyperkeratosis	0	0	0	1 (1.0)	7** (1.1)	10** (1.6)	10†† (3.0)
Sebaceous Gland, Hyperplasia	0	0	0	0	9** (1.9)	10** (2.8)	10†† (3.0)
Epidermis, Ulcer	0	0	0	1 (1.0)	1 (3.0)	7** (2.6)	8†† (3.0)
Inflammation, Chronic Active	0	0	1 (1.0)	4* (1.0)	9** (1.4)	10** (2.3)	10†† (2.7)
Dermis, Fibrosis	0	0	0	0	7** (1.1)	10** (2.4)	10†† (2.4)
Hair Follicle, Hyperplasia	0	0	0	0	9** (1.3)	10** (2.0)	9†† (1.8)
Female							
Number Examined Microscopically	10	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	4* (1.0)	7** (1.7)	10** (2.1)	10** (2.8)	10†† (3.0)
Epidermis, Hyperkeratosis	0	0	1 (1.0)	5* (1.0)	10** (1.2)	10** (2.5)	10†† (3.0)
Sebaceous Gland, Hyperplasia	0	0	0	4* (1.0)	9** (1.8)	10** (2.9)	10†† (3.0)
Epidermis, Ulcer	0	0	0	1 (2.0)	1 (3.0)	4* (2.3)	10†† (3.1)
Inflammation, Chronic Active	0	0	0	7** (1.0)	10** (1.2)	10** (2.4)	10†† (2.4)
Dermis, Fibrosis	0	0	0	1 (1.0)	7** (1.1)	8** (1.8)	10†† (2.1)
Hair Follicle, Hyperplasia	0	0	0	1 (1.0)	9** (1.7)	10** (2.8)	10†† (3.6)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
†† Significantly different (P≤0.01) from the untreated control group by the Fisher exact test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

In the kidney, the incidences of renal tubule degeneration were significantly increased in 50% and 100% males compared to those in the respective control groups (Tables 5 and A1). In addition, the incidences of renal tubule granular casts and hyaline droplet accumulation were significantly increased in males in the 25% or greater groups compared to those in the respective control groups; the severity of these lesions increased with increasing dose. The severity of nephropathy in 50% and 100% males was mild compared to minimal in both control groups and the remaining dose groups. Renal tubule degeneration was a subtle lesion graded as minimal in all dose groups and was characterized by cell swelling, mild hypereosinophilia and sloughing of the tubule epithelial cells. Rare, sloughed degenerate epithelial cells were present in the lumen of the renal cortical tubules, of which the proximal convoluted tubules were primarily affected and the distal tubules were affected to a lesser extent. The sloughed cells were shrunken and had scant eosinophilic cytoplasm and condensed dark nuclei (Plate 11). Renal tubule granular casts consisted of variable dilation of the renal tubules along the corticomedullary junction with a lightly eosinophilic, granular material and occasionally mixed with cellular debris (Plates 12 and 13). The severity of granular casts was graded as minimal if there were 10 or fewer casts in the transverse section of the kidney and 20 or fewer in the longitudinal section; mild if there were 11 to 20 casts in the transverse section and 21 to 49 in the longitudinal section; moderate if there were 21 to 49 casts in the transverse section and 50 or more in the longitudinal section; and marked when there were 50 or more casts each in the transverse and longitudinal sections. Hyaline droplet accumulation was characterized by variable amounts of granular to globular, intracytoplasmic eosinophilic, proteinaceous material within the epithelial cells of the proximal tubules. Severity of hyaline droplet accumulation was graded according to the character and amount of accumulated material in the tubule epithelial cells. In controls and males dosed with 6.25% or 12.5% cedarwood oil, the granules were fine and round (Plates 14 and 15), whereas in males dosed with 50% or 100% cedarwood oil, the material appeared as larger, variable-sized round to irregular globules (Plates 16 and 17). Nephropathy consisted of a few scattered renal tubule epithelial cell regeneration foci. The affected tubules had increased numbers of tubular epithelial cells that had basophilic cytoplasm compared to the eosinophilic cytoplasm of unaffected tubules. The basement membrane surrounding these tubules tended to be slightly thickened.

In the bone marrow, the incidences of hyperplasia were significantly increased in 100% males and females compared to those in the untreated control groups (Tables 5, A1, and A2). Bone marrow hyperplasia was characterized by increased numbers of hematopoietic cells (primarily granulocytes) within the marrow cavity and was considered to be secondary to the lesions observed in the skin at the site of application.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Male							
Kidney[a]	10	10	10	10	10	10	10
Renal Tubule, Degeneration[b]	2 (1.0)[c]	4 (1.0)	5 (1.0)	6 (1.0)	7 (1.0)	10** (1.0)	10†† (1.0)
Renal Tubule, Casts Granular	0	0	0	0	5* (1.0)	10** (1.6)	10†† (3.5)
Accumulation, Hyaline Droplet	0	0	0	0	10** (1.1)	10** (1.9)	10†† (3.0)
Nephropathy	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (2.0)	10 (2.0)
Bone Marrow	10	10	10	10	10	10	10
Hyperplasia	0	0	0	0	0	1 (1.0)	7†† (1.1)
Female							
Bone Marrow	10	10	9	10	10	10	10
Hyperplasia	0	0	0	0	2 (1.0)	2 (1.0)	7†† (1.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
†† Significantly different ($P \leq 0.01$) from the untreated control group by the Fisher exact test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-MONTH STUDY IN MICE

Due to the severities of lesions in the skin at the site of application, all males and females in the 100% groups, one male and one female each in the 50% groups, and one male in the 12.5% group were euthanized during weeks 10, 11, and 12, respectively; all other male and female mice survived to the end of the study (Table 6). The final mean body weights of 25% and 50% males and 12.5% or greater females and the mean body weight gains of 12.5% or greater males and females were significantly less than those of the vehicle control groups (Table 6 and Figure 2). Test article-related clinical observations included irritation, thickening, and ulceration of the skin at the site of application in most of the dosed mice.

The hematology data for mice are presented in Table B2. Similar to the rat study, increases in total and differential white blood cell counts occurred. The leukon changes were characterized primarily by small (20% to 30%) increases in the total white blood cell counts of 25% and 50% females and two- to threefold increases in neutrophil counts in 25% female and 50% male and female groups. Also similar to the rat study was the presence of a decreased erythron. In the mice, however, the evidence was more consistent and widespread involving decreases of all three estimators of the circulating red cell mass (i.e., the hematocrit values, hemoglobin concentrations, and erythrocyte counts) in 12.5% males and 25% and 50% males and females. In addition, the severity of the erythron decrease (approximately 13%) in the 50% groups was more than twice that of the 100% groups (approximately 5%).

There were no changes in the number of sperm and spermatids, sperm motility, or testis and epididymis weights of dosed males (Table D4). There were no estrous cycle changes in dosed females (Tables D5 and D6; Figure D2). Under the conditions of this study, cedarwood oil applied dermally did not exhibit the potential to be a reproductive toxicant in male or female B6C3F1/N mice.

TABLE 6
Survival and Body Weights of Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

Concentration (%)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
Untreated Control	10/10	21.9 ± 0.4	39.7 ± 1.3	17.8 ± 1.1	
Vehicle Control	10/10	21.7 ± 0.3	36.9 ± 0.8	15.2 ± 0.6	
6.25	10/10	21.7 ± 0.3	36.0 ± 0.6	14.4 ± 0.5	98
12.5	9/10[c]	22.1 ± 0.4	35.1 ± 1.1	13.1 ± 0.9*	95
25	10/10	22.1 ± 0.5	33.1 ± 0.9**	11.0 ± 0.7**	90
50	9/10[d]	22.1 ± 0.4	31.9 ± 0.7**	9.7 ± 0.6**	86
100	0/10[e]	—	—	—	—
Female					
Untreated Control	10/10	18.1 ± 0.2	34.9 ± 0.9	16.8 ± 0.9	
Vehicle Control	10/10	17.8 ± 0.3	34.7 ± 1.1	16.9 ± 1.0	
6.25	10/10	18.0 ± 0.4	34.0 ± 0.9	16.1 ± 0.7	98
12.5	10/10	17.9 ± 0.2	30.5 ± 0.7**	12.6 ± 0.7**	88
25	10/10	17.8 ± 0.5	28.1 ± 0.6**	10.3 ± 0.6**	81
50	9/10[d]	18.3 ± 0.4	30.2 ± 0.4**	11.9 ± 0.5**	87
100	0/10[e]	—	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test.
** $P \leq 0.01$
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Week of death: 12
[d] Week of death: 11
[e] Due to the severity of skin lesions, all 100% mice were euthanized during week 10.

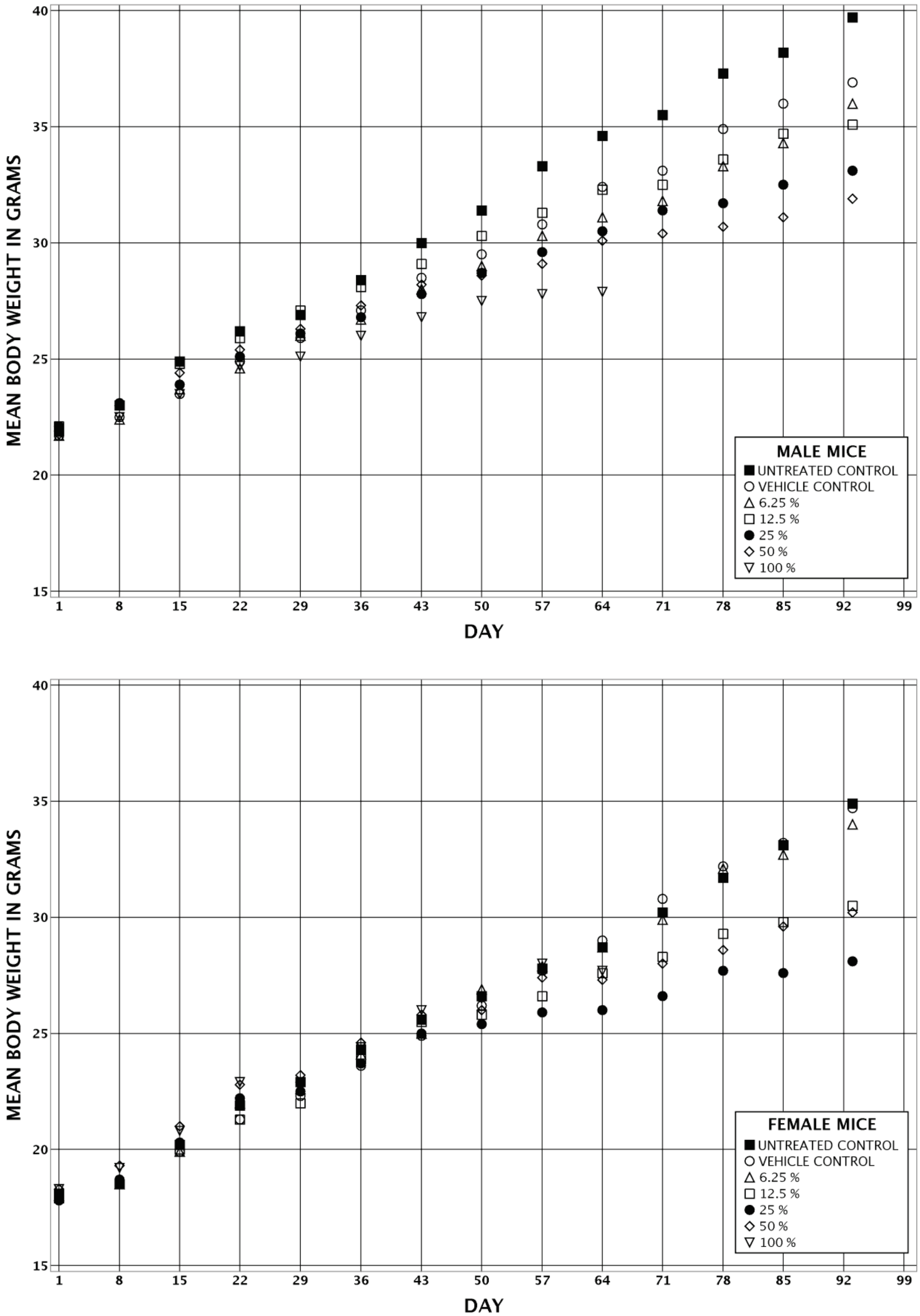


FIGURE 2
Growth Curves for Mice Administered Cedarwood Oil Dermally for 3 Months

Absolute liver weights of 50% males and females and relative liver weights of all dosed groups of males and females were significantly greater than those of the vehicle control groups (Tables 7 and C2). The absolute kidney weight of 50% females and relative kidney weights of 50% males and 12.5% or greater females were significantly greater than those of the vehicle control groups. However, the kidney weight changes in males are most likely attributable to overall body weight changes. Absolute thymus weights of 25% and 50% males and 12.5% or greater females were significantly less than those of the vehicle controls.

TABLE 7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Dermal Study of Cedarwood Oil[a,b]

	Vehicle Control	6.25%	12.5%	25%	50%
n	10	10	9	10	9
Male					
Necropsy body wt	36.9 ± 0.8	36.0 ± 0.6	35.1 ± 1.1	33.1 ± 0.9**	31.9 ± 0.7**
R. Kidney					
Absolute	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0.29 ± 0.01
Relative	7.94 ± 0.12	8.16 ± 0.22	8.51 ± 0.21	8.35 ± 0.26	9.03 ± 0.14**
Liver					
Absolute	1.66 ± 0.04	1.76 ± 0.04	1.81 ± 0.05	1.77 ± 0.06	1.98 ± 0.04**
Relative	45.06 ± 0.58	48.85 ± 1.18**	51.51 ± 0.57**	53.44 ± 1.06**	62.12 ± 1.21**
Thymus					
Absolute	0.048 ± 0.004	0.050 ± 0.002	0.044 ± 0.003	0.040 ± 0.001*	0.038 ± 0.002*
Relative	1.306 ± 0.077	1.374 ± 0.057	1.232 ± 0.063	1.224 ± 0.041	1.205 ± 0.073
Female					
Necropsy body wt	34.7 ± 1.1	34.0 ± 0.9	30.5 ± 0.7**	28.1 ± 0.6**	30.2 ± 0.4**
R. Kidney					
Absolute	0.19 ± 0.01	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.24 ± 0.01**
Relative	5.58 ± 0.18	5.94 ± 0.13	6.67 ± 0.18**	7.07 ± 0.15**	7.86 ± 0.16**
Liver					
Absolute	1.58 ± 0.05	1.65 ± 0.02	1.62 ± 0.03	1.60 ± 0.04	2.05 ± 0.05**
Relative	45.61 ± 0.79	48.75 ± 0.79*	53.24 ± 0.77**	57.10 ± 1.02**	67.80 ± 0.82**
Thymus					
Absolute	0.061 ± 0.004	0.068 ± 0.004	0.051 ± 0.002*	0.051 ± 0.003*	0.045 ± 0.002**
Relative	1.759 ± 0.087	1.990 ± 0.096	1.679 ± 0.063	1.802 ± 0.076	1.506 ± 0.070

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] Due to the severity of skin lesions, all 100% mice were euthanized during week 10.

The incidences of epidermal hyperplasia, hyperkeratosis, and ulcer and chronic active inflammation, dermal fibrosis, hair follicle hyperplasia, and sebaceous gland hyperplasia were significantly increased in most groups of males and females (Tables 8, A3, and A4).

TABLE 8
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Mice in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%[a]
Male							
Number Examined Microscopically	10	9	10	10	10	10	10
Epidermis, Hyperplasia[b]	0	0	10** (2.6)[c]	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.0)
Epidermis, Hyperkeratosis	0	0	7** (2.1)	10** (2.9)	10** (3.0)	10** (2.9)	10†† (2.9)
Epidermis, Ulcer	0	0	2 (4.0)	8** (4.0)	10** (3.8)	9** (3.7)	10†† (3.9)
Inflammation, Chronic Active	0	0	10** (2.4)	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.2)
Dermis, Fibrosis	0	0	3 (2.7)	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.0)
Hair Follicle, Hyperplasia	0	0	10** (2.6)	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.0)
Sebaceous Gland, Hyperplasia	0	0	10** (1.3)	10** (2.3)	10** (2.6)	10** (2.3)	10†† (2.0)
Female							
Number Examined Microscopically	10	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	10** (2.1)	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.0)
Epidermis, Hyperkeratosis	0	0	2 (1.5)	10** (2.7)	10** (3.0)	10** (3.0)	10†† (3.0)
Epidermis, Ulcer	0	0	2 (3.0)	6** (3.7)	9** (4.0)	10** (4.0)	10†† (4.0)
Inflammation, Chronic Active	0	0	10** (2.2)	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.0)
Dermis, Fibrosis	0	0	2 (1.0)	10** (2.9)	10** (3.0)	10** (3.0)	10†† (3.0)
Hair Follicle, Hyperplasia	0	0	3 (2.3)	10** (3.0)	10** (3.0)	10** (3.0)	10†† (3.0)
Sebaceous Gland, Hyperplasia	1 (1.0)	0	4* (1.3)	10** (2.0)	10** (2.2)	10** (2.0)	10†† (2.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
†† Significantly different ($P \leq 0.01$) from the untreated control group by the Fisher exact test
[a] Due to the severity of skin lesions, all 100% mice were euthanized during week 10.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

In general, the site of application lesions that occurred in mice were morphologically similar to those observed in the rat study. Hair follicle hyperplasia was characterized by increased numbers of cross-sectional hair follicle profiles in association with thickening of the follicular epithelium that was an extension of the hyperplastic change that occurred in the epidermis. The associated sebaceous glands were variably enlarged due to increases in size and numbers of alveolar cells (sebaceous gland hyperplasia).

In the liver, the incidences of hepatocyte glycogen depletion were significantly increased in males and females in the 12.5% or greater groups compared to those in the respective control groups (Tables 9, A3, and A4). The large, coalescing, perinuclear clear spaces (consistent with glycogen accumulation) present in the cytoplasm of hepatocytes of the controls were absent from the cytoplasm of the hepatocytes of dosed mice having the lesion. The cytoplasm of hepatocytes in the dosed mice appeared homogeneously eosinophilic or granular and contained basophilic floccular material. This was especially prominent in the 100% groups. With decreasing dose, the cytoplasm of hepatocytes appeared similar to that of the 100% groups although the clear cytoplasmic spaces were increasingly apparent but not as prominent or frequent as those in the control groups.

Compared to incidences in the respective control groups, the incidences of bone marrow myeloid cell hyperplasia were significantly increased in all dosed groups of males and females (except 6.25% females) and the incidences of hematopoietic cell proliferation in the spleen were significantly increased in all dosed groups of mice (Tables 9, A3, and A4). With the exceptions of 6.25% males and 25% females, the incidences of lymphoid hyperplasia were significantly increased in the mandibular lymph node of all dosed groups. Lymphoid hyperplasia occurred in the axillary lymph nodes of all mice, except one male in the 100% groups; one female in the 50% group also had axillary lymph node hyperplasia. Occurrences of these lesions were considered secondary to the lesions observed in the skin at the site of application. Bone marrow myeloid cell hyperplasia was characterized by increased numbers of hematopoietic cells (primarily granulocytes) within the marrow cavity of dosed animals. Splenic hematopoietic cell proliferation was characterized by expansion of the red pulp by myeloid and erythroid precursors and varying numbers of megakaryocytes. Lymphoid hyperplasia in the lymph nodes was characterized by increased numbers of lymphocytes in the lymphoid follicles and follicular and paracortical zones.

In the thymus, the incidences of atrophy were significantly increased in 25% or greater males compared to those in the respective controls (Tables 9 and A3). Atrophy consisted of depletion of thymocytes in the cortex of the thymus.

In the kidney, the incidence of nephropathy was significantly increased in 100% males compared to that in the untreated controls (Tables 9 and A3). Nephropathy consisted of sparsely scattered, focal areas of renal tubules lined by increased numbers of epithelial cells that had basophilic cytoplasm compared to surrounding normal tubules that were lined by epithelial cells that had eosinophilic cytoplasm.

TABLE 9
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%[a]
Male							
Liver[b]	10	10	10	10	10	10	10
Hepatocyte, Depletion Glycogen[c]	0	0	0	6** (1.8)[d]	9** (2.2)	10** (3.0)	10†† (3.0)
Bone Marrow	10	10	10	10	10	10	10
Myeloid Cell, Hyperplasia	0	0	5* (1.0)	10** (1.6)	10** (2.1)	10** (2.8)	10†† (4.0)
Spleen	10	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	0	6** (2.0)	10** (2.3)	10** (2.5)	10** (3.0)	10†† (2.9)
Lymph Node, Mandibular	10	10	10	10	9	10	10
Hyperplasia, Lymphoid	0	0	1 (3.0)	6** (3.0)	5* (2.6)	9** (3.2)	8†† (3.4)
Lymph Node	—[e]	—	—	—	—	—	9
Axillary, Hyperplasia, Lymphoid							9 (3.9)
Thymus	10	10	10	10	10	10	10
Atrophy	0	0	0	0	5* (1.8)	10** (1.7)	10†† (1.8)
Kidney	10	10	10	10	10	10	10
Nephropathy	0	2 (1.0)	0	1 (1.0)	2 (1.0)	3 (1.3)	5† (1.0)
Female							
Liver	10	10	10	10	10	10	10
Hepatocyte, Depletion Glycogen	0	0	0	5* (1.4)	9** (2.1)	10** (2.5)	10†† (3.5)
Bone Marrow	10	10	10	10	10	10	10
Myeloid Cell, Hyperplasia	0	0	2 (1.5)	9** (1.4)	10** (3.0)	10** (3.0)	10†† (4.0)
Spleen	10	10	10	10	10	10	10
Hematopoietic Cell Proliferation	4 (2.0)	2 (2.0)	9** (2.1)	10** (2.7)	10** (2.6)	10** (3.0)	10†† (3.0)
Lymph Node, Mandibular	10	10	10	10	10	10	10
Hyperplasia, Lymphoid	0	1 (3.0)	6* (2.7)	6* (3.0)	5 (3.6)	6* (3.5)	10†† (3.4)
Lymph Node	—	—	—	—	1	2	10
Axillary, Hyperplasia, Lymphoid					0	1 (4.0)	10 (3.6)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
† Significantly different (P≤0.05) from the untreated control group by the Fisher exact test
†† P≤0.01
[a] Due to the severity of skin lesions, all 100% mice were euthanized during week 10.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Tissue was not assessed in this group; the axillary lymph node is not a protocol required tissue and is collected and evaluated microscopically only when grossly abnormal.

GENETIC TOXICOLOGY

Cedarwood oil (0.33 to 333 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, or TA102 when tested with or without 10% induced rat liver S9 metabolic activation enzymes (Table E1). *In vivo*, no significant increases in micronucleated erythrocytes (normochromatic erythrocytes; NCEs) were observed in blood samples from male B6C3F1/N mice following 3 months of dermal exposure to cedarwood oil (6.25% to 50%) (Table E2). In female B6C3F1/N mice treated with cedarwood oil for 3 months, small increases in the frequencies of micronucleated erythrocytes were seen at the two highest doses (25% and 50%), but the mean value for micronucleated erythrocytes in each of these two treatment groups was not significantly elevated over the mean value in the vehicle control group. Because the trend test was significant (P=0.011), the results of the micronucleus assay in female mice were judged to be equivocal. No significant alterations in the percentage of micronucleated reticulocytes (polychromatic erythrocytes; PCEs) were seen in male or female mice, suggesting that cedarwood oil applied dermally did not induce bone marrow toxicity.

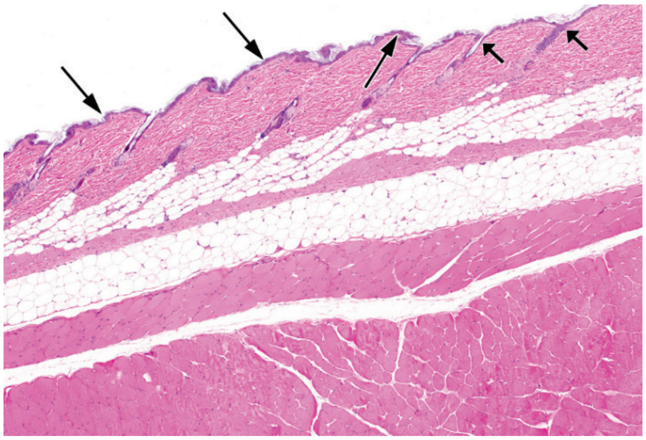


PLATE 1
Normal skin (site of application) of a vehicle control female F344/N rat in the 3-month dermal study of cedarwood oil. The epidermis (long arrows) is composed of a single layer of epithelial cells, and hair follicles are few and evenly spaced (short arrows). H&E

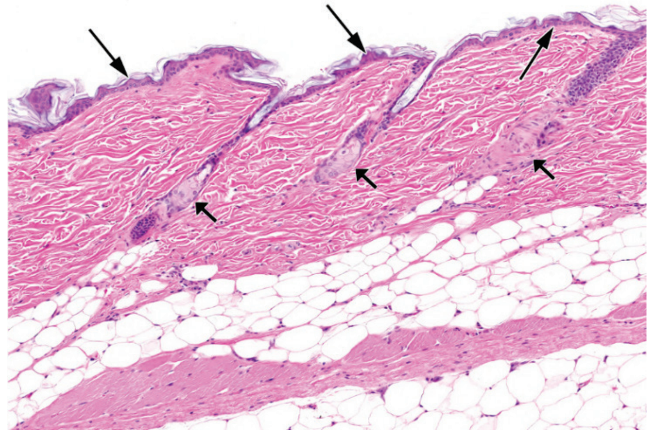


PLATE 2
Higher magnification of Plate 1. Note the epidermis (long arrows) and hair follicles with associated sebaceous glands (short arrows). H&E

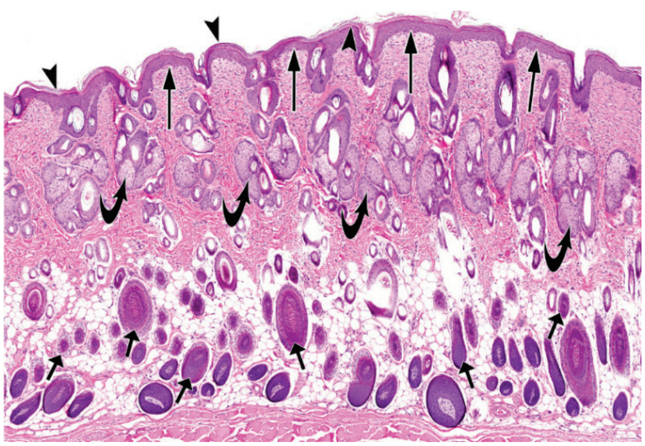


PLATE 3
Hyperplasia and hyperkeratosis of the epidermis (site of application) in the skin of a female F344/N rat administered 100% cedarwood oil dermally for 3 months. Compared to Plate 1, the epidermis (long arrows) and overlying keratin layer (arrowheads) are prominently thickened. Note that there are increased numbers of hair follicle profiles (hair follicle hyperplasia) within the dermis and subcutis (short arrows), and that the number and size of the associated sebaceous glands (curved arrows) are increased (sebaceous gland hyperplasia). H&E

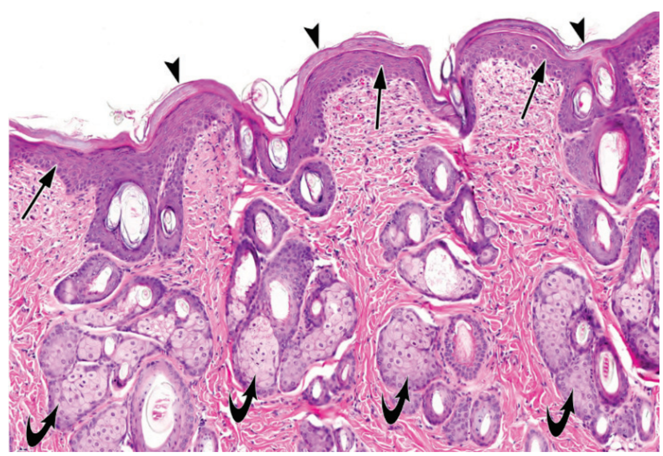


PLATE 4
Higher magnification of Plate 3. Note the thickened (hyperplastic) epidermis (long arrows), hyperkeratosis (arrowheads), and sebaceous gland hyperplasia (curved arrows). H&E

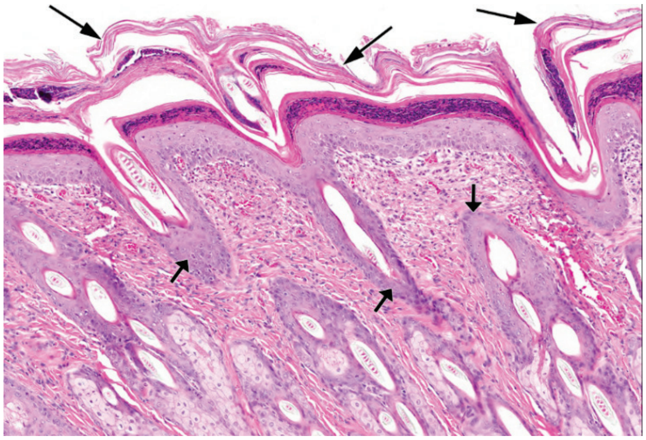


PLATE 5
Epidermal hyperkeratosis in the skin (site of application) of a female F344/N rat administered 100% cedarwood oil dermally for 3 months. There is prominent thickening of the keratin layer (long arrows) that contains accumulations of degenerate neutrophils. Note that as an extension of epidermal hyperplasia, the epithelium of the hair follicles is also hyperplastic (short arrows). Note also the infiltrates of inflammatory cells in the dermis. H&E

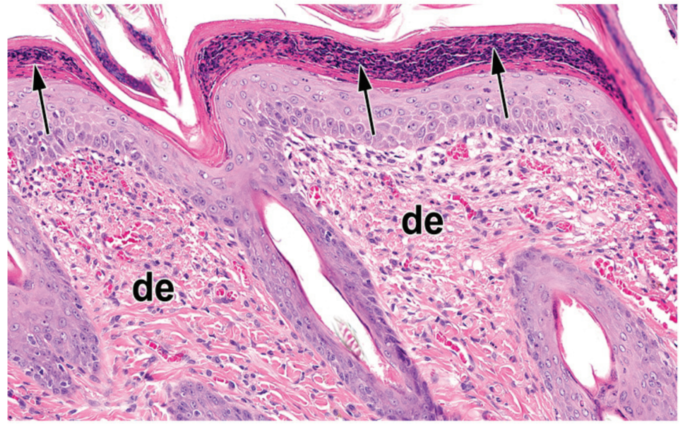


PLATE 6
Higher magnification of Plate 5. Note dense accumulations of degenerate neutrophils within the hyperplastic keratin layer (arrows) and infiltrates of inflammatory cells within the dermis (de). H&E

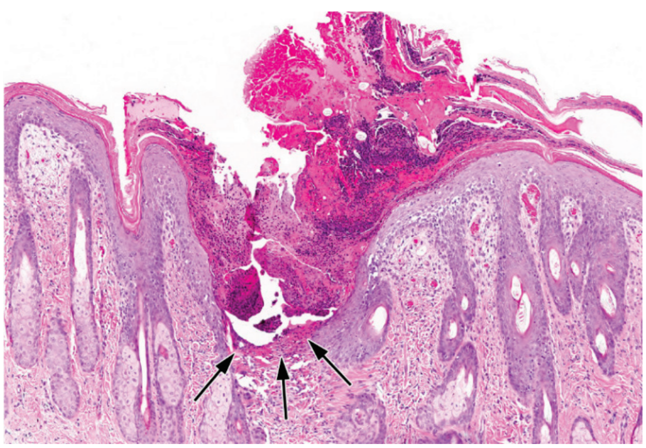


PLATE 7
Focal epidermal ulcer in the skin (site of application) of a female F344/N rat administered 100% cedarwood oil dermally for 3 months. Note focal complete loss of the epidermis (arrows) with accumulation of serum protein mixed with necrotic cellular debris at the site of ulceration. Note also epidermal, hair follicle, and sebaceous gland hyperplasia. H&E

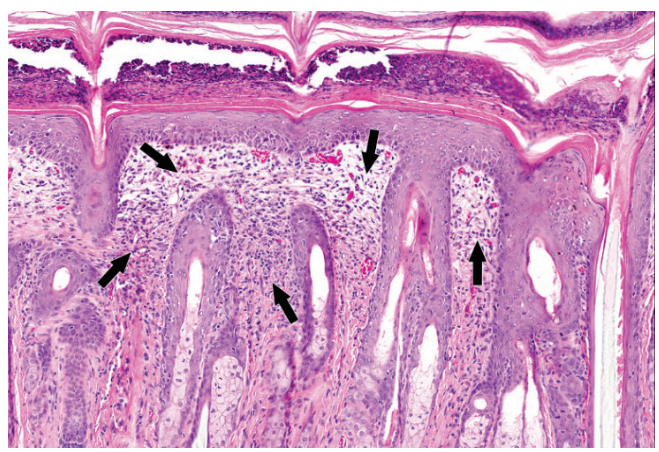


PLATE 8
Marked chronic active inflammation in the skin (site of application) of a female F344/N rat administered 100% cedarwood oil dermally for 3 months. Note a mixed inflammatory cell infiltrate within the dermis (arrows) and dense accumulation of degenerate neutrophils within the thickened keratin layer. H&E

PLATE 9
Higher magnification of Plate 8. Note degenerate neutrophils within the hyperplastic keratin layer (arrows) and infiltrates of inflammatory cells within the dermis (de). H&E

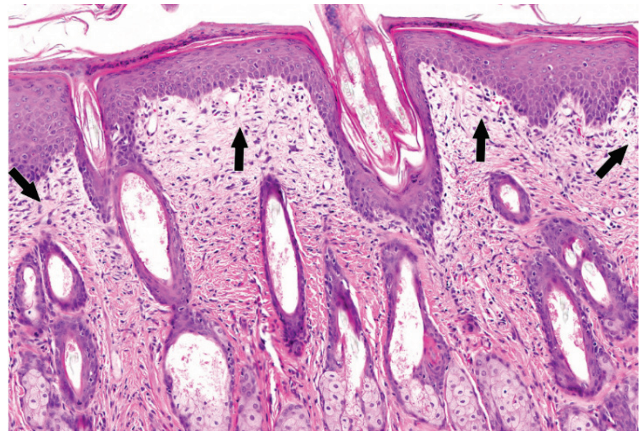


PLATE 10
Dermal fibrosis in the skin (site of application) of a female F344/N rat administered 100% cedarwood oil dermally for 3 months. Note the fibrotic tissue within the papillary dermis (arrows). H&E

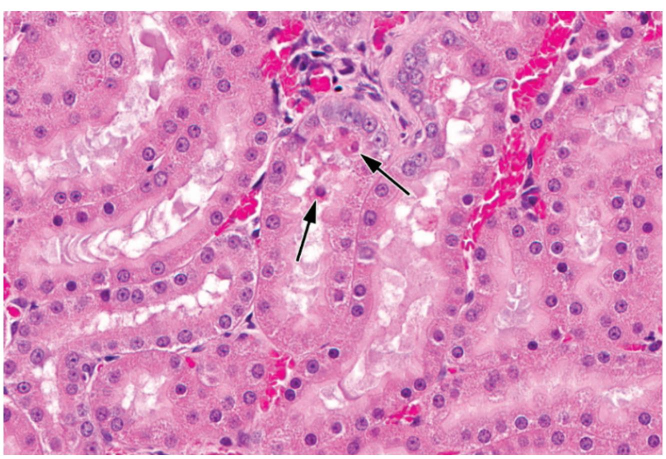


PLATE 11
Renal tubule degeneration in the kidney of a male F344/N rat administered 100% cedarwood oil dermally for 3 months. Note sloughed, shrunken, degenerate epithelial cells within the lumen of a renal tubule (arrows). H&E

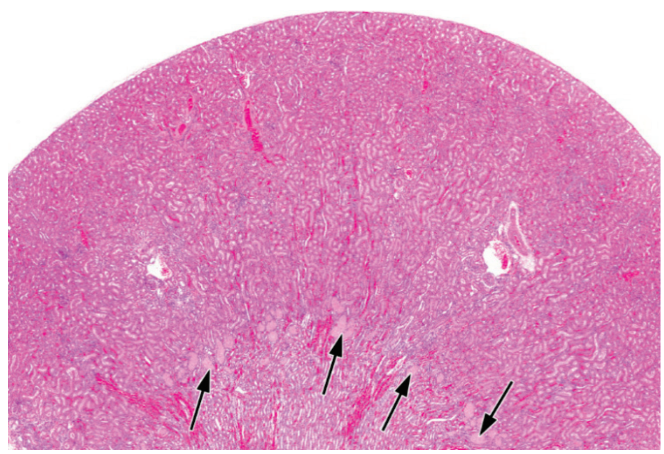


PLATE 12
Granular casts in the kidney of a male F344/N rat administered 100% cedarwood oil dermally for 3 months. The renal tubules along the corticomedullary junction (arrows) are occluded and distended by eosinophilic, granular material (casts). H&E

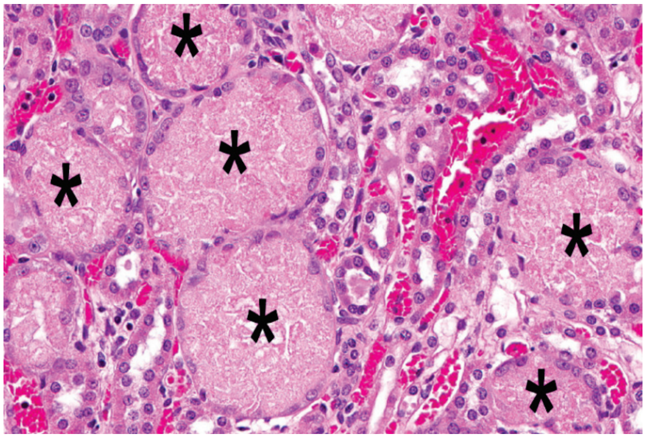


PLATE 13
Higher magnification of Plate 12 showing several renal tubules that are occluded and distended by eosinophilic, granular casts (asterisks). H&E

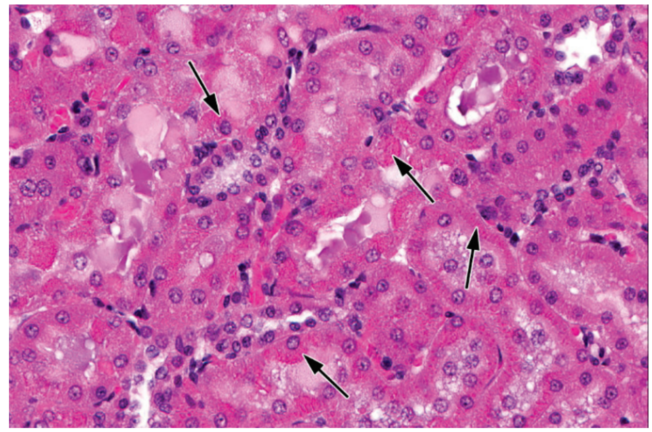


PLATE 14
Normal kidney of a vehicle control male F344/N rat in the 3-month dermal study of cedarwood oil showing uniformly fine, eosinophilic (hyaline) droplets (arrows) within the cytoplasm of several renal tubule epithelial cells. H&E

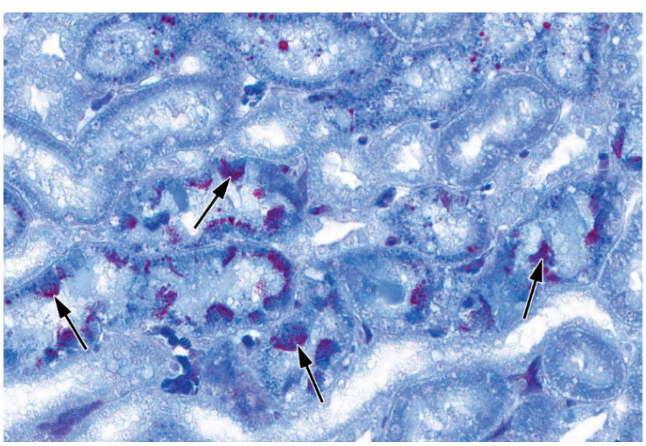


PLATE 15
Normal kidney of a vehicle control male F344/N rat in the 3-month dermal study of cedarwood oil stained with the Mallory-Heidenhain stain. This special stain for protein highlights the morphology of the fine, eosinophilic (hyaline) droplets (arrows) within the cytoplasm of several renal tubule epithelial cells. Mallory-Heidenhain

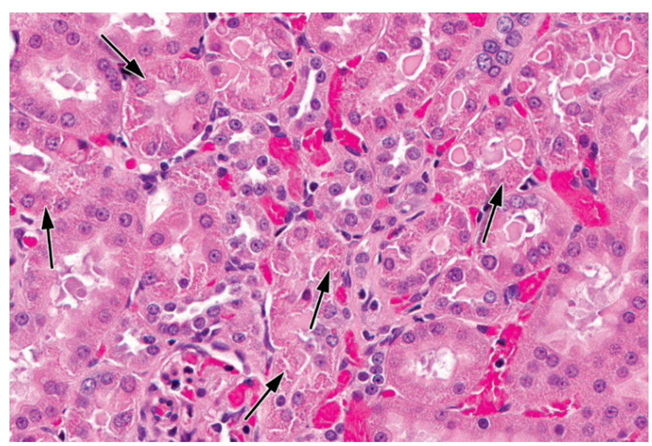


PLATE 16
Kidney of a male F344/N rat administered 100% cedarwood oil dermally for 3 months. Compared to Plate 14, there are larger, variable-sized, round to irregular eosinophilic (hyaline) droplets (arrows) within the cytoplasm of several renal tubule epithelial cells. H&E

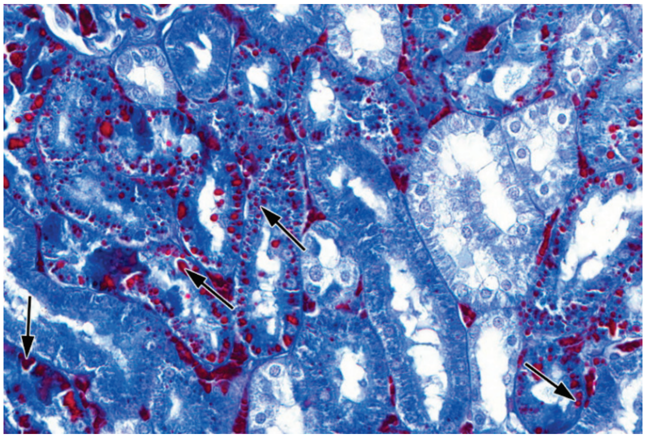


PLATE 17
Kidney of a male F344/N rat administered 100% cedarwood oil dermally for 3 months stained with the Mallory-Heidenhain stain for protein. Compared to Plate 15, note the morphology of the larger, variable-sized, round to irregular eosinophilic (hyaline) droplets (arrows) within the cytoplasm of several renal tubule epithelial cells. Mallory-Heidenhain

DISCUSSION

Virginia cedarwood oil has widespread use as a fragrance in personal and household products and as an active ingredient in the cedar balls and wooden blocks that are used as moth and bug repellants; the alcohols and terpenes from cedarwood oil have been considered Generally Recognized As Safe by the FDA as a food additive (*Ullmann's*, 1985; USEPA, 2009; 21 CFR, § 172.515). As a result of increasing widespread human exposure and the lack of toxicity data, cedarwood oil was nominated by the National Cancer Institute for subchronic toxicity studies. Given the widespread use and large production volume of Virginia cedarwood oil (hereafter referred to as cedarwood oil), this cedarwood oil product was chosen as the test article for the studies in this report. The dermal route of exposure was chosen for the current studies based on the pattern of use in humans. Due to a lack of information on the levels of exposure in the literature, the concentrations selected for these studies were untreated control; 0% (vehicle control), 6.25%, 12.5%, 25%, or 50% (with 95% ethanol as the vehicle); and 100% (neat; the highest dose possible) cedarwood oil. The untreated control group was included in addition to the 0% vehicle control group, because the 100% cedarwood oil dosing solution contained no dosing vehicle (i.e., 95% ethanol). These applied concentrations corresponded to approximately 31.25 to 500 mg cedarwood oil/kg body weight in the rat and 125 to 2,000 mg/kg in the mouse. The main toxicity targets of cedarwood oil were the skin at the site of application and the kidney.

Dosing with cedarwood oil for 3 months in rats resulted in severe skin lesions (e.g., irritation, thickening, and ulceration of the skin) at the site of application in all dosed groups, which resulted in the early termination of two male rats in the 100% group. Due to reduced survival of mice in the 100% groups and the increased incidences and severities of skin lesions at lower doses, mice appeared to be more sensitive to dermal application of cedarwood oil than rats. Though the mice appear to be more sensitive, this could be attributed to the application of a greater amount of the test article, resulting in an overall higher equivalent mg/kg dose. There was evidence of general treatment-related increases in absolute and/or relative kidney weights in the male rats. Additionally, there were general treatment-related decreases in absolute thymus weights in both sexes of both species.

During the 3-month studies, the most significant toxic response to cedarwood oil administration occurred in the skin at the site of application in rats and mice. In general, this was accompanied by microscopic lesions of minimal to moderate severities in the rats and moderate to marked severities in the mice and included epidermal hyperplasia, hyperkeratosis, and ulceration. There were also significant increases in the incidences of hair follicle and sebaceous gland hyperplasia in male and female rats in most dosed groups, and considerable increases in the incidences and severities of the lesions in mice. Incidences of chronic active inflammation increased with increasing dose in the rats and were graded minimal to mild; incidences and severities of this lesion were greater in mice than rats. Chronic active inflammation was characterized by the infiltration of neutrophils and macrophages into the dermis with occasional extensions into the hypodermis. Most data reported from studies of skin irritation or sensitization resulting from cedarwood oil exposure are conflicting and limited because of the low number of subjects evaluated. The majority of the studies testing the potential for skin irritation or sensitivity following acute exposure to cedarwood oil used low concentrations and did not find sensitization or irritation in the small numbers of animals or humans tested (Roe and Field, 1965; RIFM, 1974; Frosch *et al*., 1995). However, some of the studies did show that application of full strength cedarwood oil to rabbits for 24 hours induced moderate skin irritation (RIFM, 1974). In humans, a study examining the skin irritation potential of cedarwood oil was performed over a 24- to 72-hour period and found no evidence of skin irritation in the following groups: 0.2% (148 subjects), 2.5% (30 subjects), or 20% (29 subjects) (Fujii *et al*., 1972).

In the current studies, there were multiple indications of renal injury following dermal administration of cedarwood oil. The 3-month dermal treatment resulted in dose-dependent increases in relative kidney weights in both male and female rats. In male rats, administration of cedarwood oil resulted in the accumulation of hyaline droplets within the epithelial cells of the proximal tubules; incidences and severities of the lesion increased with increasing dose. Exacerbation of the dose-related formation and accumulation of these hyaline droplets was further verified by Mallory-Heidenhain staining. Additionally, there were pronounced granular casts within the luminal compartment of the renal tubules along the corticomedullary junction. The granular casts formed as a result of the accumulation of cellular debris within the tubules, and their presence indicated prior injury and death of the renal tubule epithelium. There was also evidence of significant renal tubule degeneration in male rats at the higher doses, which was characterized by cellular swelling, mild hypereosinophilia, and sloughing of the renal cortical tubule epithelial cells. Although the incidences of renal tubule degeneration increased with increasing dose, the lesions were subtle and given minimal severity grades in all groups. Spontaneous chronic progressive nephropathy occurs commonly in the kidneys of male, but not female, F344/N rats, and evidence of treatment-related nephropathy can be established if there is a dose-dependent increase in the severities of the lesion. In the current study, nephropathy was characterized by the presence of renal tubule epithelial regeneration foci that increased in severity in the 50% and 100% groups.

The incidences of these nonneoplastic kidney lesions may be related to α2u-globulin nephropathy, a renal syndrome that specifically occurs in male F344/N rats and has been linked to the occurrence of tubular neoplasms (Swenberg, 1993). This syndrome is characterized by several key histologic events that are thought to be secondary to toxicity, and include multifocal tubule regeneration, tubule protein casts, thickening of the tubule and glomerular basement membrane, interstitial fibrosis, and chronic inflammatory cell infiltration (Doi *et al*., 2007). This mechanism has been demonstrated in 3-month studies with several structurally diverse chemicals, including α-pinene, decalin, and propylene glycol mono-*t*-butyl ether (Doi *et al*., 2007); the incidences and severities of these histologic lesions following cedarwood oil exposure were not as marked as with these other compounds. The lesions observed in the current study meet some of the criteria for α2u-globulin-associated nephropathy in male rat renal carcinogenesis established by the United States Environmental Protection Agency (USEPA, 1991) and the International Agency for Research on Cancer (IARC, 1999). However, some of the other criteria used by these Agencies, such as measurement of α2u-globulin within the hyaline droplets, were not investigated in the current study. Though the possibility of an α2u-globulin mechanism of renal toxicity exists, the occurrence of these effects was limited to the two highest doses (50% and 100%) tested in the current study. There were also increases in kidney weights in female mice, indicating the possibility for another mechanism of toxicity.

In the current studies, there was some indication of a potential liver effect from administration of cedarwood oil in both rats and mice. Significant liver effects were more pronounced in mice, which could be attributed to the mice receiving a higher dose on a mg/kg basis. There were significant increases in relative liver weights at higher doses in male and female rats, whereas all dosed groups of mice had increases in relative liver weights. Chemical-induced increased liver weight has been associated with induction of drug-metabolizing enzymes, and is not uncommon in toxicologic studies. Drug-metabolizing enzymes such as the cytochrome P450s are often associated with liver weight increases greater than 20% and with evidence of hepatocellular hypertrophy, although hypertrophy may not always be present (Amacher *et al*., 1998). Cedrene, a major chemical constituent of cedarwood oil, has been shown *in vivo* to produce substantive increases in ethylmorphine *N*-demethylase activities and cytochrome P450 content in comparison to controls, with minimal differences due to routes of administration (Hashimoto *et al*., 1972). Another study exhibited that animals housed on cedar shavings had increased incidences of spontaneous tumors of the liver as well as a highly significant reduction in barbiturate sleeping time, an indication of the induction of liver drug metabolizing enzymes including cytochrome P450 (Sabine, 1975). Conversely, in more recent *in vitro* studies, the constituents cedrol, β-cedrene, and thujopsene were found to inhibit the activities of CYP2B6 and CYP3A4 in human liver microsomes (Jeong *et al*., 2014). Additionally, in the current study, microscopic evaluation of hepatocytes within male and female mice revealed increased incidences and severities of glycogen depletion at all doses greater than 6.25% that generally increased with increasing dose. Hepatocyte glycogen depletion was characterized by the lack of large, coalescing, perinuclear clear spaces within the hepatocyte cytoplasm in the dosed animals compared to the controls. Glycogen storage in hepatocytes acts as a cellular energy reserve, and in fasting conditions, these glycogen stores may be depleted. Hepatocyte glycogen depletion in the mice administered cedarwood oil may be related to the decrease in body weight and reduced food intake, which is supported by the inverse relationship between the decreased body weights and depletion of their glycogen stores.

Significantly increased incidences of nonneoplastic lesions occurred in the bone marrow, lymph nodes, and spleen of most dosed groups of mice. In the bone marrow, this occurred in the incidences of myeloid cell hyperplasia in all dosed groups of mice, except for 6.25% females. Lymphoid hyperplasia occurred in both the mandibular and axillary lymph nodes, however, lymphoid hyperplasia in the axillary lymph nodes occurred only in the 100% group. Significantly increased incidences and severities of splenic hematopoietic cell proliferation occurred in all dosed groups of mice. These effects are indicative of an inflammatory reaction to the dermal application of cedarwood oil and are considered to be secondary to the severe skin lesions at the application site. This is supported by alterations in clinical pathology parameters, which included increases in leukon alongside decreases in erythron. This increase in the total and differential white blood cell counts (specifically the neutrophils) was consistent with an inflammatory leukogram secondary to the dermal irritation and inflammation. The decreased erythron with no alterations in mean cell volume or mean cell hemoglobin concentration is consistent with an anemia of chronic disease also secondary to the inflammation at the site of application (Weiss and Goodnough, 2005).

Dermal exposure to cedarwood oil resulted in decreases in the absolute and relative thymus weights of rats and mice. On a mg/kg body weight basis, the mice received doses of cedarwood oil that were approximately four times higher than the rats, which could be why the mice had higher incidences of decreased thymus weights across more doses than did the rats. In mice, significant decreases in absolute thymus weights were observed in 25% and 50% males and 12.5% or greater females compared to the vehicle controls. Thymus weight decreases in the male mice were accompanied by thymic atrophy, which consisted of thymocyte depletion in the cortex of the thymus. In rats, absolute thymus weights were significantly decreased in 25% and 100% males and 100% females. The thymus is the most sensitive of the lymphoid organs in response to stress, and stress-related decreases in weight due to lymphocyte depletion may be seen within hours of exposure (Everds *et al.*, 2013). The thymus effects observed in the current studies in rats and mice are consistent with stress-related organ effects that are a common secondary finding in toxicity studies, and can show dose proportionality.

Cedarwood oil was not found to be mutagenic in any of the *Salmonella typhimurium* strains tested *in vitro* or in an *in vivo* micronucleus test in the peripheral blood of male mice. However, increased micronucleated erythrocyte counts were judged to be equivocal in female mice following 3 months of dermal exposure to cedarwood oil, due to a significant positive trend in the absence of significant dosed group increases relative to controls. In addition, there was no evidence of treatment-related alterations within the micronucleated reticulocyte population in either male or female mice, suggesting that bone marrow toxicity is not induced by dermal application of cedarwood oil.

Under the conditions of the 3-month dermal studies with Virginia cedarwood oil, there were treatment-related lesions in male and female rats and mice. Skin (at the site of application) and kidney were the major targets from administration of cedarwood oil in both rats and mice. Additionally, the liver and the thymus were considered secondary targets of cedarwood oil administration as a result of the skin effects at the site of application in both rats and mice. The most sensitive measures of cedarwood oil administration in each species and sex were: increased incidences of skin (site of application) lesions in male [lowest-observed-effect-level (LOEL) = 12.5%; approximately equivalent to 62.5 mg/kg] and female (LOEL = 6.25%; approximately equivalent to 31.25 mg/kg) rats and increased incidences of skin (site of application) lesions (LOEL = 6.25%; approximately equivalent to 124 mg/kg) in male and female mice.

REFERENCES

Abifadel, R., Mortureux, P., Perromat, M., Ducombs, G., and Taieb, A. (1992). Contact sensitivity to flavourings and perfumes in atopic dermatitis. *Contact Dermatitis* **27**, 43-46.

Acevedo, Z. (1979). Abortion in early America. *Women Health* **4**, 159-167.

Adams, R.P. (1989). *Identification of Essential Oils by Ion Trap Mass Spectrometry*. Academic Press, Inc., San Diego, CA.

Adams, R.P. (1991). Cedar Wood Oil-Analyses and Properties. In *Modern Methods of Plant Analysis New Series, Essential Oils and Waxes* (H.F. Linsken and J.F. Jackson, Eds.), Vol. 12, pp. 159-173. Springer-Verlag, New York.

Adams, R.P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy.* Allured Publishing Corporation, Carol Stream, IL.

The Aldrich Library of Infrared Spectra (1981). 3rd ed. (C.J. Pouchert, Ed.), Vol. 3, Spectrum 66B. Sigma-Aldrich Chemical Company, Milwaukee, WI.

Amacher, D.E., Schomaker, S.J., and Burkhardt, J.E. (1998). The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. *Food Chem. Toxicol*. **36**, 831-839.

Barnard, D.R. (1999). Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J. Med. Entomol*. **36**, 625-629.

Bellanato, J., and Hidalgo, A. (1971). *Infrared Analysis of Essential Oils*, pp. 125-126. Heyden and Son Limited, London.

Belsito, D., Bickers, D., Bruze, M., Calow, P., Dagli, M.L., Fryer, A.D., Greim, H., Miyachi, Y., Saurat, J.H., and Sipes, I.G. (2013). A toxicological and dermatological assessment of alkyl cyclic ketones when used as fragrance ingredients. *Food Chem. Toxicol*. **62**, S1-S44.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Ciesla, W.M. (1998). Non-wood forest products from conifers. In *Non-Wood Forest Products, Vol. 12*. Food and Agriculture Organization of the United Nations. Rome, Italy.

Code of Federal Regulations (CFR) **21**, Part 58.

Code of Federal Regulations (CFR) **21**, § 172.515.

Code of Federal Regulations (CFR) **40**, § 152.25.

Coppen, J.J.W. (1995). Flavours and fragrances of plant origin. In *Non-Wood Forest Products, Vol. 1*. Food and Agriculture Organization of the United Nations. Rome, Italy.

Cosmetic Ingredient Review (CIR) (2001). Final report on the safety assessment of Juniperus communis extract, Juniperus oxycedrus extract, Juniperus oxycedrus tar, Juniperus phoenicea extract, and Juniperus viginiana extract. *Int. J. Toxicol.* **20**, 41-56.

Craig, A.M., Karchesy, J.J., Blythe, L.L., González-Hernández, M.dP., Swan, L.R. (2004). Toxicity studies on western juniper oil (*Juniperus occidentalis*) and Port-Orford-cedar oil (*Chamaecyparis lawsoniana*) extracts utilizing local lymph node and acute dermal irritation assays. *Toxicol. Lett*. **154**, 217-224.

Curtis, C.F., Lines, J.D., Ilumba, J., Callaghan, A., Hill, N., and Karimzad, M.A. (1987). The relative efficacy of repellents against mosquito vectors of disease. *Med. Vet. Entomol*. **1**, 109-119.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Doi, A.M., Hill, G., Seely, J., Hailey, J.R., Kissling, G., and Bucher, J.R. (2007). Alpha 2u-globulin nephropathy and renal tumors in national toxicology program studies. *Toxicol. Pathol*. **35**, 533-540.

Dunford, N.T., Hiziroglu, S., and Holcomb, R. (2007). Effect of age on the distribution of oil in Eastern red cedar tree segments. *Bioresour. Technol*. **98**, 2636-2640.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Eller, F.J., Vander Meer, R.K., Behle, R.W., Flor-Weiler, L.B., and Palmquist, D.E. (2014). Bioactivity of cedarwood oil and cedrol against arthropod pests. *Environ. Entomol*. **43**, 762-766.

Everds, N.E., Snyder, P.W., Bailey, K.L., Bolon, B., Creasy, D.M., Foley, G.L., Rosol, T.J., and Sellers, T. (2013). Interpreting stress responses during routine toxicity studies: A review of the biology, impact, and assessment. *Toxicol. Pathol*. **41**, 560-614.

Federal Register (1992). Modification in voluntary filing of cosmetic product ingredient and cosmetic raw material composition statements. Vol. 57, No. 18, pp. 3128-3130. Food and Drug Administration, Washington, DC.

Franz, H., Frank, R., Rytter, M., and Haustein, U.-F. (1998). Allergic contact dermatitis due to cedarwood oil after dermatoscopy. *Contact Dermatitis* **38**, 182-183.

Frosch, P.J., Pilz, B., Andersen, K.E., Burrows, D., Camarasa, J.G., Dooms-Goossens, A., Ducombs, G., Fuchs, T., Hannuksela, M., Lachapelle, J.M., Lahti, A., Maibach, H.I., Menné, T., Rycroft, R.J.G., Shaw, S., Wahlberg, J.E., White, I.R., and Wilkinson, J.D. (1995). Patch testing with fragrances: Results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* **33**, 333-342.

Fujii, T., Furukawa, S., and Suzuki, S. (1972). Studies on compounded perfumes for toilet goods. On the non-irritative compounded perfumes for soaps [in Japanese, English abstract]. *Yukagaku* **21**, 904-908.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Girard, D.M., and Sager, D.B. (1987). The use of Markov chains to detect subtle variation in reproductive cycling. *Biometrics* **43**, 225-234.

Gosselin, R.E., Smith, R.P., and Hodge, H.C. (1984). *Clinical Toxicology of Commercial Products*, 5th ed., p. II-231. Williams and Wilkins, Baltimore/London.

Hashimoto, M., Davis, D.C., and Gillette, J.R. (1972). Effect of different routes of administration of cedrene on hepatic drug metabolism. *Biochem. Pharmacol*. **21**, 1514-1517.

Hazardous Substances Data Bank (HSDB) (2014). Cedarwood Oil. TOXNET: Toxicology Data Network. National Library of Medicine. <http://toxnet.nlm.nih.gov/hsdb.htm> Accessed July 2014.

Hong, J.Y., Lee, B.H., Kim, T.H., Hong, J., Lee, K.M., Yoo, S.D., and Lee, H.S. (2013). GC-MS/MS method for the quantification of α-cedrene in rat plasma and its pharmacokinetic application. *J. Sep. Sci*. **36**, 3558-3562.

International Agency for Research on Cancer (IARC) (1999). Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis (C.C. Capen, E. Dybing, J.M. Rice, and J.D. Wilbourn, Eds). IARC Scientific Publications No. 147. IARC, Lyon, France.

International Organization for Standardization (ISO) (2004). Oil of cedarwood, Virginian (*Juniperus virginiana* L.). ISO 4724:2004€. Prepared by Technical Committee ISO/TC54, *Essential Oils*.

Jeong, H.-U., Kwon, S.-S., Kong, T.Y., Kim, J.H., and Lee, H.S. (2014). Inhibitory effects of cedrol, β-cedrene, and thujopsene on cytochrome P450 enzyme activities in human live microsomes. *J. Toxicol. Environ. Health A* **77**, 1522-1532.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kim, T.H., Yoo, S.D., Lee, H.S., Lee, K.M., Seok, S.H., Kim, M.G., Jung, B.H., Kim, M.G., and Shin, B.S. (2015). In vivo absorption and disposition of α-cedrene, a sesquiterpene constituent of cedarwood oil, in female and male rats. *Drug Metab. Pharmacokinet*. **30**, 168-173.

Kligman, A.M. (1966). The identification of contact allergens by human assay. 3. The maximization test: A procedure for screening and rating contact sensitizers. *J. Invest. Dermatol*. **47**, 393-409.

Lapczynski, A., Isola, D.A., Christian, M.S., Diener, R.M., and Api, A.M. (2006). Evaluation of the developmental toxicity of acetyl cedrene. *Int. J. Toxicol*. **25**, 423-428.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol*. **14**, 513-522.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol*. **10**, 71-80.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 1073. Merck and Company, Rahway, NJ.

Merory, J. (1968). *Food Flavorings: Composition, Manufacture, and Use*, 2nd ed. AVI Publishing Company, Inc., Westport, CT.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983), unpublished provisional data as of July 1, 1990. NIOSH, Cincinnati, OH.

Ogata, A., Ando, H., Kubo, Y., Nagasawa, A., Ogawa, H., Yasuda, K., and Aoki, N. (1999). Teratogenicity of thujaplicin in ICR mice. *Food Chem. Toxicol*. **37**, 1097-1104.

Research Institute for Fragrance Materials (RIFM) (1974). Fragrance Raw Materials Monographs: Cedarwood Oil Virginia. *Food Cosmet. Toxicol*. **12**, 845-846.

Roe, F.J.C., and Field, W.E.H. (1965). Chronic toxicity of essential oils and certain other products of natural origin. *Food Cosmet. Toxicol*. **3**, 311-323.

Sabine, J.R. (1975). Exposure to an environment containing the aromatic red cedar, *Juniperus virginiana*: Procarcinogenic, enzyme-inducing, and insecticidal effects. *Toxicology* **5**, 221-235.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Singh, D., Rao, S.M., and Tripathi, A.K. (1984). Cedarwood oil as a potential insecticidal agent against mosquitoes. *Naturwissenschaften* **71**, 265-266.

Swenberg, J.A. (1993). α2u-Globulin nephropathy: Review of the cellular and molecular mechanisms involved and their implications for human risk assessment. *Environ. Health Perspect*. **101**, 39-44.

Takao, Y., Kuriyama, I., Yamada, T., Mizoguchi, H., Yoshida, H., and Mizushina, Y. (2012). Antifungal properties of Japanese cedar essential oil from waste wood chips made from used sake barrels. *Mol. Med. Rep*. **5**, 1163-1168.

Ullmann's Encyclopedia of Industrial Chemistry (1985). 5th ed. (W. Gerhartz, Ed.), p. 220. VCH Publishers, Deerfield Beach, FL.

United States Environmental Protection Agency (USEPA) (1991). Alpha$_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. EPA/625/3-91/019F. U.S. Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency (USEPA) (1993). Reregistration Eligibility Decision (RED) Cedarwood Oil. <http://epa.gov/oppsrrd1/REDs/old_reds/cedarwood_oil.pdf> Accessed June 2014.

United States Environmental Protection Agency (USEPA) (2009). Wood Oils and Gums Summary Document Registration Review: Initial Docket. <http://www.epa.gov/oppsrrd1/registration_review/wood-oils/> Accessed June 2014.

United States Environmental Protection Agency (USEPA) (2013). Cedarwood oil. <http://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do?>

Weiss, G., and Goodnough, L.T. (2005). Anemia of chronic disease. *N. Engl. J. Med*. **352**, 1011-1023.

Welch, K.D., Cook, D., Gardner, D.R., Parsons, C., Pfister, J.A., and Panter, K.E. (2013). A comparison of the abortifacient risk of western juniper trees in Oregon. *Rangelands* **35**, 40-44.

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biom. Bull.* **1**, 80-83.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen*. **19** (Suppl. 21), 2-141.

APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Early deaths							
Moribund							2
Survivors							
Terminal euthanasia	10	10	10	10	10	10	8
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Esophagus	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, rectum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Parasite metazoan	1 (10%)						1 (10%)
Intestine small, duodenum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)		1 (10%)		1 (10%)	
Hepatodiaphragmatic nodule	1 (10%)	1 (10%)					
Inflammation	8 (80%)	8 (80%)	10 (100%)	10 (100%)	7 (70%)	7 (70%)	9 (90%)
Necrosis, focal							1 (10%)
Pancreas	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Atrophy		1 (10%)					1 (10%)
Infiltration cellular, mononuclear cell	4 (40%)	5 (50%)					3 (30%)
Salivary glands	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Submandibular gland, vacuolization cytoplasmic	5 (50%)	2 (20%)					4 (40%)
Stomach, forestomach	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, glandular	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Glands, ectasia		2 (20%)					
Cardiovascular System							
Blood vessel	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	10 (100%)	10 (100%)					10 (100%)
Epicardium, hyperplasia							1 (10%)
Endocrine System							
Adrenal cortex	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Angiectasis	4 (40%)	2 (20%)					
Adrenal medulla	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Islets, pancreatic	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)	1 (10%)					
Parathyroid gland	(9)	(9)	(0)	(0)	(0)	(0)	(10)
Pituitary gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Cyst		1 (10%)					

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Endocrine System (continued)							
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Ultimobranchial cyst			2 (20%)				
General Body System							
None							
Genital System							
Epididymis	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell		1 (10%)					
Inflammation	1 (10%)	2 (20%)					2 (20%)
Preputial gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, focal, chronic		1 (10%)					3 (30%)
Prostate	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation	2 (20%)						
Seminal vesicle	(10)	(10)	(0)	(1)	(0)	(0)	(10)
Testes	(10)	(10)	(0)	(1)	(0)	(0)	(10)
Germinal epithelium, right, atrophy				1 (100%)			
Hematopoietic System							
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia						1 (10%)	7 (70%)
Lymph node, mesenteric	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Spleen	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Hematopoietic cell proliferation		1 (10%)					
Thymus	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Integumentary System							
Mammary gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Skin	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hair follicle, site of application, hyperplasia					9 (90%)	10 (100%)	9 (90%)
Sebaceous gland, site of application, hyperplasia					9 (90%)	10 (100%)	10 (100%)
Site of application, inflammation, chronic active			1 (10%)	4 (40%)	9 (90%)	10 (100%)	10 (100%)
Site of application, dermis, fibrosis					7 (70%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperkeratosis				1 (10%)	7 (70%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperplasia			2 (20%)	4 (40%)	10 (100%)	10 (100%)	9 (90%)
Site of application, epidermis, ulcer				1 (10%)	1 (10%)	7 (70%)	8 (80%)

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Musculoskeletal System							
Bone	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System							
Brain	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Respiratory System							
Lung	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Hemorrhage							1 (10%)
Infiltration cellular, histiocyte	2 (20%)	1 (10%)					2 (20%)
Inflammation	2 (20%)	5 (50%)					4 (40%)
Metaplasia, osseous	5 (50%)	5 (50%)					4 (40%)
Pigmentation, hemosiderin							1 (10%)
Serosa, inflammation, granulomatous							1 (10%)
Nose	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic active							2 (20%)
Trachea	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Special Senses System							
Eye	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Harderian gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Urinary System							
Kidney	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Accumulation, hyaline droplet					10 (100%)	10 (100%)	10 (100%)
Infiltration cellular, mononuclear cell	1 (10%)	1 (10%)			1 (10%)		1 (10%)
Mineralization	9 (90%)	4 (40%)	7 (70%)	9 (90%)	8 (80%)	8 (80%)	8 (80%)
Nephropathy	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, casts granular					5 (50%)	10 (100%)	10 (100%)
Renal tubule, degeneration	2 (20%)	4 (40%)	5 (50%)	6 (60%)	7 (70%)	10 (100%)	10 (100%)
Urinary bladder	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)	1 (10%)					1 (10%)
Serosa, mineralization, focal							1 (10%)

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal euthanasia	10	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Esophagus	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, rectum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Parasite metazoan		1 (10%)					
Intestine small, duodenum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)						
Hepatodiaphragmatic nodule	2 (20%)	1 (10%)					
Inflammation	10 (100%)	9 (90%)	10 (100%)	9 (90%)	8 (80%)	5 (50%)	7 (70%)
Mesentery	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Mineralization			1 (100%)				
Necrosis			1 (100%)				
Fat, inflammation, granulomatous, chronic active			1 (100%)				
Pancreas	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Atrophy							1 (10%)
Infiltration cellular, mononuclear cell	7 (70%)	4 (40%)					3 (30%)
Salivary glands	(10)	(10)	(0)	(0)	(10)	(10)	(10)
Sublingual gland, ectopic tissue							1 (10%)
Submandibular gland, ectopic tissue							1 (10%)
Submandibular gland, vacuolization cytoplasmic	3 (30%)	4 (40%)					1 (10%)
Stomach, forestomach	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, glandular	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Cardiovascular System							
Blood vessel	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	10 (100%)	9 (90%)					10 (100%)
Inflammation, chronic active	1 (10%)						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Endocrine System							
Adrenal cortex	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular	1 (10%)						
Infiltration cellular, mononuclear cell		1 (10%)					
Mineralization		1 (10%)					
Adrenal medulla	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Islets, pancreatic	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Parathyroid gland	(9)	(9)	(0)	(0)	(0)	(0)	(10)
Pituitary gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Cyst	2 (20%)	2 (20%)					1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mononuclear cell		2 (20%)					1 (10%)
Ultimobranchial cyst		3 (30%)	2 (20%)		3 (30%)	3 (30%)	2 (20%)
General Body System							
None							
Genital System							
Clitoral gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)						
Inflammation, focal, chronic active	1 (10%)						
Inflammation, chronic active	1 (10%)						
Ovary	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Left, cyst	1 (10%)						
Uterus	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Hematopoietic System							
Bone marrow	(10)	(10)	(9)	(10)	(10)	(10)	(10)
Hyperplasia					2 (20%)	2 (20%)	7 (70%)
Lymph node, mesenteric	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Spleen	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Thymus	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Integumentary System							
Mammary gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Integumentary System (continued)							
Skin	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hair follicle, site of application, hyperplasia				1 (10%)	9 (90%)	10 (100%)	10 (100%)
Sebaceous gland, site of application, hyperplasia				4 (40%)	9 (90%)	10 (100%)	10 (100%)
Site of application, inflammation, chronic active				7 (70%)	10 (100%)	10 (100%)	10 (100%)
Site of application, dermis, fibrosis				1 (10%)	7 (70%)	8 (80%)	10 (100%)
Site of application, epidermis, hyperkeratosis			1 (10%)	5 (50%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperplasia			4 (40%)	7 (70%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, ulcer				1 (10%)	1 (10%)	4 (40%)	10 (100%)
Musculoskeletal System							
Bone	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System							
Brain	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Respiratory System							
Lung	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, histiocyte		1 (10%)					1 (10%)
Inflammation	3 (30%)	2 (20%)					2 (20%)
Metaplasia, osseous	1 (10%)	1 (10%)					
Nose	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic active							1 (10%)
Vacuolization cytoplasmic		1 (10%)					
Trachea	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)						
Special Senses System							
Eye	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Harderian gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular							1 (10%)
Urinary System							
Kidney	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infarct, focal, chronic	1 (10%)						
Mineralization	5 (50%)	5 (50%)					6 (60%)
Nephropathy		1 (10%)					1 (10%)
Urinary bladder	(10)	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	5 (50%)	2 (20%)					

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Early deaths							
Moribund				1		1	10
Survivors							
Terminal euthanasia	10	10	10	9	10	9	
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Esophagus	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Gallbladder	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Intestine large, cecum	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Intestine large, colon	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Intestine large, rectum	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Intestine small, duodenum	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Intestine small, ileum	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Intestine small, jejunum	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation						1 (10%)	
Infarct							1 (10%)
Inflammation, chronic active	8 (80%)	7 (70%)	6 (60%)	8 (80%)	7 (70%)	2 (20%)	1 (10%)
Necrosis, focal		1 (10%)				1 (10%)	2 (20%)
Hepatocyte, depletion glycogen				6 (60%)	9 (90%)	10 (100%)	10 (100%)
Mesentery	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Fat, fibrosis						1 (100%)	
Fat, necrosis						1 (100%)	
Pancreas	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Salivary glands	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Inflammation, chronic active	1 (10%)						
Stomach, forestomach	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Stomach, glandular	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Cardiovascular System							
Blood vessel	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Heart	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Endocrine System							
Adrenal cortex	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Accessory adrenal cortical nodule	1 (10%)	2 (20%)				2 (20%)	
Adrenal medulla	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Islets, pancreatic	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Parathyroid gland	(10)	(9)	(0)	(1)	(0)	(10)	(10)
Cyst		1 (11%)					1 (10%)
Pituitary gland	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Thyroid gland	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Ectopic thymus	1 (10%)						
Follicle, degeneration						1 (10%)	2 (20%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
General Body System							
None							
Genital System							
Coagulating gland	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Epididymis	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Preputial gland	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Prostate	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Seminal vesicle	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Testes	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Hematopoietic System							
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Myeloid cell, hyperplasia			5 (50%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Lymph node	(0)	(0)	(0)	(0)	(0)	(0)	(9)
Axillary, hyperplasia, lymphoid							9 (100%)
Lymph node, mandibular	(10)	(10)	(10)	(10)	(9)	(10)	(10)
Hyperplasia, lymphoid			1 (10%)	6 (60%)	5 (56%)	9 (90%)	8 (80%)
Lymph node, mesenteric	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation			6 (60%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					5 (50%)	10 (100%)	10 (100%)
Ectopic parathyroid gland		1 (10%)				1 (10%)	
Integumentary System							
Skin	(10)	(9)	(10)	(10)	(10)	(10)	(10)
Hair follicle, site of application, dilatation						1 (10%)	1 (10%)
Hair follicle, site of application, hyperplasia			10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Sebaceous gland, site of application, hyperplasia			10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, inflammation, chronic active			10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, dermis, fibrosis			3 (30%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperkeratosis			7 (70%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperplasia			10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, ulcer			2 (20%)	8 (80%)	10 (100%)	9 (90%)	10 (100%)
Musculoskeletal System							
Bone	(10)	(10)	(0)	(1)	(0)	(10)	(10)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Nervous System							
Brain	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Respiratory System							
Lung	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Metaplasia, osseous	1 (10%)						
Thrombosis		1 (10%)					
Nose	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Trachea	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Special Senses System							
Eye	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Harderian gland	(10)	(10)	(0)	(1)	(0)	(10)	(10)
Urinary System							
Kidney	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active							1 (10%)
Nephropathy		2 (20%)		1 (10%)	2 (20%)	3 (30%)	5 (50%)
Urinary bladder	(10)	(10)	(0)	(1)	(0)	(10)	(10)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Early deaths							
Moribund						1	10
Survivors							
Terminal euthanasia	10	10	10	10	10	9	
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Esophagus	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Gallbladder	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Intestine large, cecum	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Intestine large, colon	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Intestine large, rectum	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Intestine small, duodenum	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Intestine small, ileum	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Intestine small, jejunum	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Diverticulum						1 (10%)	
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Infarct							1 (10%)
Inflammation, chronic active	10 (100%)	10 (100%)	8 (80%)	9 (90%)	9 (90%)	10 (100%)	8 (80%)
Tension, lipidosis						1 (10%)	
Hepatocyte, depletion glycogen				5 (50%)	9 (90%)	10 (100%)	10 (100%)
Hepatocyte, necrosis							1 (10%)
Pancreas	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Salivary glands	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Stomach, forestomach	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Stomach, glandular	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Mineralization		1 (10%)					
Cardiovascular System							
Heart	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Endocrine System							
Adrenal cortex	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Accessory adrenal cortical nodule	1 (10%)						
Adrenal medulla	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Islets, pancreatic	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Parathyroid gland	(9)	(9)	(0)	(0)	(0)	(8)	(9)
Pituitary gland	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Thyroid gland	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Ectopic thymus		1 (10%)					
General Body System							
None							

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Genital System							
Clitoral gland	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Ovary	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Uterus	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Decidual reaction		1 (10%)					
Endometrium, hyperplasia, cystic	1 (10%)	2 (20%)					
Hematopoietic System							
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Myeloid cell, hyperplasia			2 (20%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Lymph node	(0)	(0)	(0)	(0)	(1)	(2)	(10)
Hyperplasia, lymphoid					1 (100%)	1 (50%)	
Axillary, hyperplasia, lymphoid						1 (50%)	10 (100%)
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid		1 (10%)	6 (60%)	6 (60%)	5 (50%)	6 (60%)	10 (100%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	4 (40%)	2 (20%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Inflammation, chronic active	1 (10%)						
Thymus	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Integumentary System							
Mammary gland	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Skin	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hair follicle, site of application, dilatation							3 (30%)
Hair follicle, site of application, hyperplasia			3 (30%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Sebaceous gland, site of application, hyperplasia	1 (10%)		4 (40%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, inflammation, chronic active			10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, dermis, fibrosis			2 (20%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperkeratosis			2 (20%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, hyperplasia			10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Site of application, epidermis, ulcer			2 (20%)	6 (60%)	9 (90%)	10 (100%)	10 (100%)
Musculoskeletal System							
Bone	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Nervous System							
Brain	(10)	(10)	(0)	(0)	(0)	(10)	(10)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 3-Month Dermal Study of Cedarwood Oil

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Respiratory System							
Lung	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Nose	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Trachea	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Special Senses System							
Eye	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Harderian gland	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Urinary System							
Kidney	(10)	(10)	(0)	(0)	(0)	(10)	(10)
Inflammation, chronic active							2 (20%)
Nephropathy						1 (10%)	
Urinary bladder	(10)	(10)	(0)	(0)	(0)	(10)	(10)

APPENDIX B
HEMATOLOGY RESULTS

TABLE B1
Hematology Data for Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
Male							
n	10	9	10	9	10	10	8
Hematocrit (%)	50.4 ± 0.6	49.2 ± 0.6	50.2 ± 0.4	48.8 ± 0.8	49.1 ± 0.6	48.5 ± 0.3	49.0 ± 0.6
Hemoglobin (g/dL)	15.9 ± 0.2	15.6 ± 0.2	15.8 ± 0.1	15.4 ± 0.2	15.6 ± 0.2	15.4 ± 0.1	15.5 ± 0.1
Erythrocytes (10^6/µL)	9.31 ± 0.12	9.12 ± 0.11	9.25 ± 0.07	9.08 ± 0.14	9.14 ± 0.11	9.04 ± 0.05	9.03 ± 0.10†
Reticulocytes (10^3/µL)	223.8 ± 6.4	237.7 ± 8.0	229.5 ± 8.6	234.7 ± 10.3	202.1 ± 8.8*	203.8 ± 8.6*	188.4 ± 10.8†
Mean cell volume (fL)	54.1 ± 0.2	54.0 ± 0.2	54.2 ± 0.2	53.8 ± 0.1	53.7 ± 0.2	53.7 ± 0.2	54.3 ± 0.1
Mean cell hemoglobin (pg)	17.1 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	17.0 ± 0.1	17.0 ± 0.1	17.0 ± 0.1	17.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.5 ± 0.2	31.7 ± 0.1	31.5 ± 0.1	31.6 ± 0.1	31.7 ± 0.1	31.6 ± 0.1	31.6 ± 0.1
Platelets (10^3/µL)	545 ± 16	579 ± 13	577 ± 20	552 ± 50	559 ± 42	578 ± 18	632 ± 18††
Leukocytes (10^3/µL)	8.16 ± 0.36	8.98 ± 0.50	8.87 ± 0.38	9.35 ± 0.42	8.89 ± 0.23	9.62 ± 0.28	10.60 ± 0.17††
Segmented neutrophils (10^3/µL)	1.07 ± 0.05	1.23 ± 0.06	1.43 ± 0.09	1.33 ± 0.08	1.35 ± 0.10	1.70 ± 0.13*	3.28 ± 0.21††
Lymphocytes (10^3/µL)	6.73 ± 0.32	7.41 ± 0.48	7.05 ± 0.33	7.62 ± 0.42	7.17 ± 0.25	7.54 ± 0.33	6.81 ± 0.26
Monocytes (10^3/µL)	0.22 ± 0.02	0.21 ± 0.02	0.25 ± 0.02	0.24 ± 0.03	0.22 ± 0.01	0.22 ± 0.01	0.35 ± 0.02††
Basophils (10^3/µL)	0.044 ± 0.005	0.068 ± 0.010	0.048 ± 0.004	0.064 ± 0.005	0.059 ± 0.003	0.060 ± 0.006	0.055 ± 0.006
Eosinophils (10^3/µL)	0.09 ± 0.01	0.06 ± 0.01	0.09 ± 0.01*	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.11 ± 0.01
Female							
n	10	8	9	9	10	7	9
Hematocrit (%)	49.3 ± 1.4	47.5 ± 0.3	48.4 ± 0.9	47.8 ± 0.9	47.1 ± 0.5	48.9 ± 1.1	46.5 ± 0.6†
Hemoglobin (g/dL)	16.1 ± 0.4	15.6 ± 0.1	15.8 ± 0.2	15.7 ± 0.2	15.6 ± 0.1	15.9 ± 0.3	15.2 ± 0.2†
Erythrocytes (10^6/µL)	8.72 ± 0.20	8.42 ± 0.07	8.50 ± 0.11	8.50 ± 0.12	8.37 ± 0.07	8.62 ± 0.16	8.34 ± 0.09
Reticulocytes (10^3/µL)	210.4 ± 7.7	202.4 ± 9.8	222.9 ± 15.2	200.9 ± 9.4	205.5 ± 7.2	187.1 ± 10.3	191.2 ± 4.5
Mean cell volume (fL)	56.5 ± 0.4	56.5 ± 0.4	56.9 ± 0.3	56.2 ± 0.4	56.2 ± 0.3	56.7 ± 0.4	55.9 ± 0.2
Mean cell hemoglobin (pg)	18.5 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.4 ± 0.1	18.6 ± 0.1	18.4 ± 0.1	18.3 ± 0.1†
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.2	32.8 ± 0.2	32.6 ± 0.2	32.8 ± 0.2	33.1 ± 0.2	32.6 ± 0.2	32.7 ± 0.2
Platelets (10^3/µL)	501 ± 47	608 ± 30	473 ± 57	464 ± 40*	504 ± 38	588 ± 30	537 ± 56
Leukocytes (10^3/µL)	6.60 ± 0.37	6.85 ± 0.57	6.96 ± 0.19	7.04 ± 0.38	6.70 ± 0.40	8.89 ± 0.33**	9.47 ± 0.35††
Segmented neutrophils (10^3/µL)	1.02 ± 0.11	1.15 ± 0.16	0.97 ± 0.07	1.22 ± 0.21	1.18 ± 0.08	1.50 ± 0.09*	2.38 ± 0.15††
Lymphocytes (10^3/µL)	5.28 ± 0.31	5.39 ± 0.45	5.68 ± 0.21	5.49 ± 0.28	5.24 ± 0.35	6.97 ± 0.30*	6.65 ± 0.29††
Monocytes (10^3/µL)	0.18 ± 0.01	0.20 ± 0.03	0.19 ± 0.01	0.19 ± 0.02	0.16 ± 0.02	0.25 ± 0.01	0.27 ± 0.03†
Basophils (10^3/µL)	0.046 ± 0.006	0.046 ± 0.006	0.038 ± 0.006	0.038 ± 0.007	0.037 ± 0.006	0.056 ± 0.008	0.056 ± 0.004
Eosinophils (10^3/µL)	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.02	0.10 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.12 ± 0.01†

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
† Significantly different ($P \leq 0.05$) from the untreated control group by Wilcoxon's rank sum test
†† $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

TABLE B2
Hematology Data for Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

	Vehicle Control	6.25%	12.5%	25%	50%
Male					
n	9	10	9	10	9
Hematocrit (%)	46.9 ± 0.8	45.4 ± 0.6	44.7 ± 0.6*	44.0 ± 0.7**	41.2 ± 0.7**
Hemoglobin (g/dL)	15.9 ± 0.3	15.5 ± 0.2	15.1 ± 0.2*	14.8 ± 0.2**	13.9 ± 0.2**
Erythrocytes (10^6/µL)	10.30 ± 0.20	9.95 ± 0.13	9.77 ± 0.12*	9.61 ± 0.12**	9.06 ± 0.16**
Reticulocytes (10^3/µL)	328.3 ± 10.9	309.3 ± 11.4	321.2 ± 9.8	319.4 ± 9.6	330.7 ± 24.6
Mean cell volume (fL)	45.5 ± 0.1	45.6 ± 0.1	45.7 ± 0.2	45.7 ± 0.2	45.5 ± 0.2
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.4 ± 0.1	15.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.0 ± 0.2	34.2 ± 0.1	33.8 ± 0.2	33.7 ± 0.2	33.7 ± 0.2
Platelets (10^3/µL)	938 ± 49	992 ± 47	1,061 ± 54	1,136 ± 37**	1,111 ± 44*
Leukocytes (10^3/µL)	5.14 ± 0.13	5.55 ± 0.29	5.82 ± 0.35	5.83 ± 0.35	6.07 ± 0.66
Segmented neutrophils (10^3/µL)	0.77 ± 0.07	0.85 ± 0.16	0.89 ± 0.08	0.85 ± 0.85	1.99 ± 0.43**
Lymphocytes (10^3/µL)	4.15 ± 0.11	4.21 ± 0.22	4.42 ± 0.21	4.54 ± 0.28	3.73 ± 0.27
Monocytes (10^3/µL)	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.10 ± 0.02
Basophils (10^3/µL)	0.014 ± 0.005	0.017 ± 0.003	0.017 ± 0.004	0.012 ± 0.002	0.016 ± 0.003
Eosinophils (10^3/µL)	0.11 ± 0.02	0.35 ± 0.04**	0.39 ± 0.09**	0.31 ± 0.06*	0.23 ± 0.03
Female					
n	10	10	10	10	8
Hematocrit (%)	46.9 ± 0.7	45.8 ± 0.6	45.2 ± 0.7	44.0 ± 0.6**	40.9 ± 0.4**
Hemoglobin (g/dL)	16.4 ± 0.2	16.0 ± 0.2	15.7 ± 0.2*	15.2 ± 0.2**	14.1 ± 0.1**
Erythrocytes (10^6/µL)	10.22 ± 0.14	9.99 ± 0.11	9.93 ± 0.15	9.70 ± 0.14**	8.92 ± 0.08**
Reticulocytes (10^3/µL)	343.6 ± 15.3	284.2 ± 8.8*	343.3 ± 21.5	307.4 ± 15.0	311.7 ± 27.2
Mean cell volume (fL)	45.9 ± 0.1	45.9 ± 0.2	45.6 ± 0.1	45.4 ± 0.1*	45.9 ± 0.2
Mean cell hemoglobin (pg)	16.0 ± 0.0	16.0 ± 0.1	15.8 ± 0.1*	15.7 ± 0.1**	15.9 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	34.9 ± 0.1	34.9 ± 0.1	34.7 ± 0.2	34.5 ± 0.1	34.5 ± 0.2
Platelets (10^3/µL)	685 ± 47	764 ± 42	887 ± 64*	960 ± 57**	1,058 ± 43**
Leukocytes (10^3/µL)	5.07 ± 0.30	4.66 ± 0.18	5.56 ± 0.36	6.23 ± 0.30*	6.98 ± 0.21**
Segmented neutrophils (10^3/µL)	0.65 ± 0.07	0.52 ± 0.03	0.80 ± 0.12	1.27 ± 0.14*	1.65 ± 0.18**
Lymphocytes (10^3/µL)	4.15 ± 0.27	3.87 ± 0.17	4.39 ± 0.30	4.58 ± 0.23	4.90 ± 0.23*
Monocytes (10^3/µL)	0.11 ± 0.02	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.12 ± 0.01
Basophils (10^3/µL)	0.011 ± 0.005	0.009 ± 0.003	0.022 ± 0.007	0.013 ± 0.004	0.036 ± 0.004**
Eosinophils (10^3/µL)	0.15 ± 0.02	0.17 ± 0.05	0.22 ± 0.04	0.26 ± 0.06	0.28 ± 0.03

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX C
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Untreated Control	Vehicle Control	6.25%	12.5%	25%	50%	100%
n	10	10	10	10	10	10	8
Male							
Necropsy body wt	323 ± 4	329 ± 4	318 ± 5	316 ± 6	308 ± 10	301 ± 8*	279 ± 7††
Heart							
Absolute	0.91 ± 0.01	0.91 ± 0.01	0.92 ± 0.02	0.91 ± 0.02	0.88 ± 0.03	0.90 ± 0.02	0.87 ± 0.02
Relative	2.83 ± 0.02	2.77 ± 0.03	2.89 ± 0.05	2.90 ± 0.04	2.87 ± 0.03	2.99 ± 0.04**	3.12 ± 0.05††
R. Kidney							
Absolute	1.09 ± 0.02	1.15 ± 0.02†	1.08 ± 0.03	1.10 ± 0.02	1.12 ± 0.04	1.19 ± 0.03	1.33 ± 0.05††
Relative	3.37 ± 0.05	3.51 ± 0.04†	3.40 ± 0.04	3.50 ± 0.07	3.63 ± 0.06	3.96 ± 0.08**	4.77 ± 0.11††
Liver							
Absolute	12.42 ± 0.37	12.75 ± 0.26	12.91 ± 0.38	12.47 ± 0.31	12.60 ± 0.49	13.07 ± 0.37	13.43 ± 0.42
Relative	38.55 ± 1.21	38.79 ± 0.58	40.58 ± 0.97	39.52 ± 0.75	40.89 ± 0.86	43.49 ± 0.84**	48.18 ± 1.31††
Lung							
Absolute	2.02 ± 0.11	1.98 ± 0.09	1.94 ± 0.11	1.93 ± 0.05	1.99 ± 0.11	0.95 ± 0.06	2.04 ± 0.10
Relative	6.26 ± 0.32	6.02 ± 0.23	6.10 ± 0.30	6.12 ± 0.15	6.42 ± 0.20	6.51 ± 1.22	7.30 ± 0.31†
R. Testis							
Absolute	1.398 ± 0.018	1.384 ± 0.014	1.376 ± 0.024	1.314 ± 0.086	1.373 ± 0.025	1.389 ± 0.025	1.341 ± 0.020
Relative	4.335 ± 0.058	4.216 ± 0.056	4.339 ± 0.107	4.144 ± 0.258	4.477 ± 0.094	4.627 ± 0.064*	4.819 ± 0.106††
Thymus							
Absolute	0.282 ± 0.013	0.282 ± 0.006	0.277 ± 0.008	0.280 ± 0.010	0.245 ± 0.011*	0.271 ± 0.014	0.223 ± 0.016†
Relative	0.875 ± 0.044	0.857 ± 0.014	0.870 ± 0.023	0.887 ± 0.033	0.798 ± 0.032	0.904 ± 0.049	0.797 ± 0.051
Female							
Necropsy body wt	200 ± 4	197 ± 2	199 ± 4	194 ± 5	193 ± 4	182 ± 4*	179 ± 3††
Heart							
Absolute	0.66 ± 0.02	0.64 ± 0.01	0.66 ± 0.02	0.65 ± 0.02	0.66 ± 0.02	0.63 ± 0.02	0.65 ± 0.02
Relative	3.33 ± 0.08	3.27 ± 0.05	3.31 ± 0.05	3.35 ± 0.07	3.41 ± 0.05	3.46 ± 0.08	3.64 ± 0.09
R. Kidney							
Absolute	0.72 ± 0.01	0.71 ± 0.02	0.73 ± 0.02	0.71 ± 0.02	0.74 ± 0.02	0.70 ± 0.02	0.75 ± 0.01
Relative	3.59 ± 0.07	3.59 ± 0.05	3.65 ± 0.04	3.63 ± 0.05	3.85 ± 0.06**	3.86 ± 0.07**	4.17 ± 0.09††
Liver							
Absolute	6.97 ± 0.18	7.02 ± 0.18	6.93 ± 0.23	7.10 ± 0.19	7.14 ± 0.18	6.80 ± 0.21	7.58 ± 0.13†
Relative	34.94 ± 0.60	35.62 ± 0.79	34.74 ± 0.55	36.60 ± 0.62	36.98 ± 0.56	37.28 ± 0.56	42.30 ± 0.42††
Lung							
Absolute	1.68 ± 0.14	1.41 ± 0.07	1.56 ± 0.13	1.55 ± 0.08	1.53 ± 0.11	1.53 ± 0.12	1.45 ± 0.08
Relative	8.44 ± 0.66	7.15 ± 0.33	7.82 ± 0.58	8.07 ± 0.50	7.89 ± 0.51	8.39 ± 0.63	8.06 ± 0.38
Thymus							
Absolute	0.279 ± 0.016	0.240 ± 0.006†	0.263 ± 0.012	0.245 ± 0.013	0.244 ± 0.008	0.225 ± 0.009	0.213 ± 0.009††
Relative	1.395 ± 0.071	1.220 ± 0.030†	1.320 ± 0.055	1.265 ± 0.064	1.262 ± 0.036	1.236 ± 0.046	1.189 ± 0.055††

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
† Significantly different ($P \leq 0.05$) from the untreated control group by *t*-test
†† $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

	Vehicle Control	6.25%	12.5%	25%	50%
Male					
n	10	10	9	10	9
Necropsy body wt	36.9 ± 0.8	36.0 ± 0.6	35.1 ± 1.1	33.1 ± 0.9**	31.9 ± 0.7**
Heart					
Absolute	0.19 ± 0.01	0.22 ± 0.01	0.23 ± 0.01*	0.23 ± 0.01**	0.25 ± 0.02**
Relative	5.15 ± 0.26	5.96 ± 0.21	6.60 ± 0.31**	7.07 ± 0.33**	7.80 ± 0.52**
R. Kidney					
Absolute	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0.29 ± 0.01
Relative	7.94 ± 0.12	8.16 ± 0.22	8.51 ± 0.21	8.35 ± 0.26	9.03 ± 0.14**
Liver					
Absolute	1.66 ± 0.04	1.76 ± 0.04	1.81 ± 0.05	1.77 ± 0.06	1.98 ± 0.04**
Relative	45.06 ± 0.58	48.85 ± 1.18**	51.51 ± 0.57**	53.44 ± 1.06**	62.12 ± 1.21**
Lung					
Absolute	0.31 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.02
Relative	8.39 ± 0.49	7.93 ± 0.37	8.31 ± 0.49	8.37 ± 0.24	8.67 ± 0.45
R. Testis					
Absolute	0.118 ± 0.003	0.117 ± 0.003	0.116 ± 0.004	0.118 ± 0.003	0.114 ± 0.004
Relative	3.188 ± 0.054	3.258 ± 0.107	3.319 ± 0.094	3.568 ± 0.087**	3.574 ± 0.107**
Thymus					
Absolute	0.048 ± 0.004	0.050 ± 0.002	0.044 ± 0.003	0.040 ± 0.001*	0.038 ± 0.002*
Relative	1.306 ± 0.077	1.374 ± 0.057	1.232 ± 0.063	1.224 ± 0.041	1.205 ± 0.073
Female					
n	10	10	10	10	9
Necropsy body wt	34.7 ± 1.1	34.0 ± 0.9	30.5 ± 0.7**	28.1 ± 0.6**	30.2 ± 0.4**
Heart					
Absolute	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01*	0.22 ± 0.01**	0.24 ± 0.01**
Relative	5.17 ± 0.17	5.54 ± 0.21	6.70 ± 0.37**	7.70 ± 0.26**	8.02 ± 0.31**
R. Kidney					
Absolute	0.19 ± 0.01	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.24 ± 0.01**
Relative	5.58 ± 0.18	5.94 ± 0.13	6.67 ± 0.18**	7.07 ± 0.15**	7.86 ± 0.16**
Liver					
Absolute	1.58 ± 0.05	1.65 ± 0.02	1.62 ± 0.03	1.60 ± 0.04	2.05 ± 0.05**
Relative	45.61 ± 0.79	48.75 ± 0.79*	53.24 ± 0.77**	57.10 ± 1.02**	67.80 ± 0.82**
Lung					
Absolute	0.30 ± 0.01	0.31 ± 0.01	0.29 ± 0.02	0.28 ± 0.01	0.27 ± 0.02
Relative	8.78 ± 0.35	9.05 ± 0.27	9.40 ± 0.46	10.09 ± 0.37	9.06 ± 0.59
Thymus					
Absolute	0.061 ± 0.004	0.068 ± 0.004	0.051 ± 0.002*	0.051 ± 0.003*	0.045 ± 0.002**
Relative	1.759 ± 0.087	1.990 ± 0.096	1.679 ± 0.063	1.802 ± 0.076	1.506 ± 0.070

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).`

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Vehicle Control	12.5%	25%	50%
n	10	10	10	10
Weights (g)				
Necropsy body wt	329 ± 4	316 ± 6	308 ± 10	301 ± 8*
L. Cauda epididymis	0.1545 ± 0.0019	0.1466 ± 0.0037	0.1495 ± 0.0072	0.1495 ± 0.0079
L. Epididymis	0.4592 ± 0.0120	0.4424 ± 0.0115	0.4502 ± 0.0171	0.4416 ± 0.0138
L. Testis	1.4565 ± 0.0142	1.4440 ± 0.0070	1.4260 ± 0.0251	1.4240 ± 0.0257
Spermatid measurements				
Spermatid heads (10^6/testis)	179.50 ± 4.85	187.63 ± 8.09	186.13 ± 10.08	197.63 ± 6.46
Spermatid heads (10^6/g testis)	138.35 ± 4.10	146.15 ± 6.60	147.21 ± 7.31	156.29 ± 4.67
Epididymal spermatozoal measurements				
Sperm motility (%)	84.7 ± 0.6	84.7 ± 0.7	83.7 ± 0.6	84.2 ± 0.8
Sperm (10^6/cauda epididymis)	115.0 ± 7.2	116.4 ± 7.7	121.4 ± 8.4	107.2 ± 9.9
Sperm (10^6/g cauda epididymis)	748.5 ± 51.7	794.9 ± 49.6	811.5 ± 36.4	716.3 ± 55.4

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE D2
Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study of Cedarwood Oil[a]

	Vehicle Control	12.5%	25%	50%
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	197 ± 2	193 ± 4	182 ± 4**	179 ± 3**
Proportion of regular cycling females[b]	10/10	10/10	10/10	10/10
Estrous cycle length (days)	5.0 ± 0.0	5.0 ± 0.0	5.1 ± 0.1	5.0 ± 0.0
Estrous stages (% of cycle)				
Diestrus	60.8	62.5	62.5	55.0
Proestrus	20.0	17.5	16.7	20.8
Estrus	19.2	19.2	19.2	22.5
Metestrus	0.0	0.0	0.0	0.0
Uncertain diagnosis	0.0	0.8	1.7	1.7

** Significantly different (P≤0.01) from the vehicle control group by Williams' test

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group were not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated no significant differences in transition probabilities among the groups.

[b] Number of females with a regular cycle/number of females cycling

Concentration (%)																			
Vehicle Control					D	P	E	D	D	D	P	E	D	D	D	P			
Vehicle Control						P	E	D	D	D	P	E	D	D	D	P	E		
Vehicle Control				D	D	P	E	D	D	D	P	E	D	D	D				
Vehicle Control			D	D	D	P	E	D	D	D	P	E	D	D					
Vehicle Control							E	D	D	D	P	E	D	D	D	P	E	D	
Vehicle Control				D	D	P	E	D	D	D	P	E	D	D	D				
Vehicle Control							E	D	D	D	P	E	D	D	D	P	E	D	
Vehicle Control					D	P	E	D	D	D	P	E	D	D	D	P			
Vehicle Control					D	P	E	D	D	D	P	E	D	D	D	P			
Vehicle Control			D	D	D	P	E	D	D	D	P	E	D	D					
12.5							E	D	D	D	P	E	D	D	D	P	E	D	
12.5				D	D	P	E	D	D	D	P	E	D	D	D				
12.5					D	P	E	D	D	D	P	E	D	D	D	P			
12.5				D	D	P	E	D	D	D	P	E	D	D	D				
12.5				D	D	P	E	D	D	D	P	E	D	D	D				
12.5						P	E	D	D	D	P	E	D	D	D	P	E		
12.5				D	D	P	E	D	D	D	P	E	D	D	D				
12.5					D	IC	E	D	D	D	P	E	D	D	D	P			
12.5			D	D	D	P	E	D	D	D	P	E	D	D					
12.5						D	D	E	D	D	D	E	E	D	D	D	P		
25			D	D	D	P	E	D	D	D	P	E	D	D					
25						P	E	D	D	D	P	E	D	D	D	P	E		
25						P	E	D	D	D	D	E	D	D	D	P	E		
25		IC	IC	D	D	P	E	D	D	D	P	E	D						
25			D	D	D	P	E	D	D	D	P	E	D	D					
25						P	E	D	D	D	D	E	D	D	D	P	E		
25			D	D	D	P	E	D	D	D	P	E	D	D					
25				D	D	P	E	D	D	D	P	E	D	D	D				
25					D	E	D	D	D	D	P	E	D	D	D	D			
25			D	D	D	P	E	D	D	D	P	E	D	D					
50							E	D	D	D	P	E	D	D	D	P	E	D	
50							E	D	D	D	P	E	D	D	D	P	E	D	
50					D	P	E	D	D	D	P	E	D	D	D	P			
50					D	P	E	D	D	D	P	E	D	D	D	P			
50							E	D	D	D	P	E	D	D	D	P	E	D	
50						P	E	D	D	D	P	E	D	D	D	P	E		
50						P	E	D	D	D	P	E	D	D	D	P	E		
50		IC	IC	D	D	P	E	D	D	D	P	E	D						
50						P	E	D	D	D	P	E	D	D	D	P	E		
50						D	P	E	D	D	D	E	E	D	D	D	P		

FIGURE D1
Vaginal Cytology Plots for Female Rats in the 3-Month Dermal Study of Cedarwood Oil
D = diestrus, P = proestrus, E = estrus, IC=insufficient number of cells to determine stage

TABLE D3
Results of Vaginal Cytology Study Using the Transition Matrix Approach for Female Rats in the 3-Month Dermal Study of Cedarwood Oil

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	0.989	
Overall Tests	12.5% vs. Vehicle Controls	0.953	
Overall Tests	25% vs. Vehicle Controls	0.683	
Overall Tests	50% vs. Vehicle Controls	0.975	
Extended Estrus	Overall	0.918	
Extended Estrus	12.5% vs. Vehicle Controls	0.604	
Extended Estrus	25% vs. Vehicle Controls	1	
Extended Estrus	50% vs. Vehicle Controls	0.604	
Extended Diestrus	Overall	0.993	
Extended Diestrus	12.5% vs. Vehicle Controls	1	
Extended Diestrus	25% vs. Vehicle Controls	0.683	
Extended Diestrus	50% vs. Vehicle Controls	0.996	N
Extended Metestrus	Overall	1	
Extended Metestrus	12.5% vs. Vehicle Controls	1	
Extended Metestrus	25% vs. Vehicle Controls	1	
Extended Metestrus	50% vs. Vehicle Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	12.5% vs. Vehicle Controls	1	
Extended Proestrus	25% vs. Vehicle Controls	1	
Extended Proestrus	50% vs. Vehicle Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	12.5% vs. Vehicle Controls	1	
Skipped Estrus	25% vs. Vehicle Controls	1	
Skipped Estrus	50% vs. Vehicle Controls	1	
Skipped Diestrus	Overall	1	
Skipped Diestrus	12.5% vs. Vehicle Controls	1	
Skipped Diestrus	25% vs. Vehicle Controls	1	
Skipped Diestrus	50% vs. Vehicle Controls	1	

[a] N means that the dosed group had a lower probability of transitioning to and/or from the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

TABLE D4
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

	Vehicle Control	6.25%	12.5%	25%
n	10	10	9	10
Weights (g)				
Necropsy body wt	36.9 ± 0.8	36.0 ± 0.6	35.1 ± 1.1	33.1 ± 0.9**
L. Cauda epididymis	0.0161 ± 0.0018	0.0143 ± 0.0009	0.0143 ± 0.0007	0.0141 ± 0.0005
L. Epididymis	0.0436 ± 0.0023	0.0423 ± 0.0015	0.0396 ± 0.0012	0.0417 ± 0.0011
L. Testis	0.1089 ± 0.0032	0.1100 ± 0.0022	0.1092 ± 0.0026	0.1094 ± 0.0025
Spermatid measurements				
Spermatid heads (10^6/testis)	21.57 ± 0.72	21.42 ± 0.75	22.85 ± 0.60	21.98 ± 0.66[b]
Spermatid heads (10^6/g testis)	210.41 ± 9.00	208.10 ± 9.26	214.88 ± 5.46	217.22 ± 5.31[b]
Epididymal spermatozoal measurements				
Sperm motility (%)	83.1 ± 0.3	81.6 ± 1.9	82.7 ± 0.6	83.9 ± 0.4
Sperm (10^6/cauda epididymis)	12.5 ± 1.4	12.6 ± 1.1	12.6 ± 1.0	13.3 ± 2.1
Sperm (10^6/g cauda epididymis)	820.3 ± 105.9	884.7 ± 70.4	886.8 ± 69.1	1,022.9 ± 121.2

** Significantly different (P≤0.01) from the vehicle control group by Williams' test
a Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
b n=9

TABLE D5
Estrous Cycle Characterization for Female Mice in the 3-Month Dermal Study of Cedarwood Oil[a]

	Vehicle Control	6.25%	12.5%	25%
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	34.7 ± 1.1	34.0 ± 0.9	30.5 ± 0.7**	28.1 ± 0.6**
Proportion of regular cycling females[b]	8/10	8/10	9/10	9/10
Estrous cycle length (days)	4.2 ± 0.14	4.5 ± 0.4	3.9 ± 0.09	4.1 ± 0.15
Estrous stages(% of cycle)				
Diestrus	31.7	30.0	32.5	26.7
Proestrus	0.0	0.0	0.0	0.0
Estrus	45.0	45.8	45.8	45.0
Metestrus	21.7	24.2	21.7	24.2
Uncertain diagnoses	1.7	0.0	0.0	4.2

** Significantly different (P≤0.01) from the vehicle control group by Williams' test
a Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated no significant differences in transition probabilities among the groups.
b Number of females with a regular cycle/number of females cycling

Concentration (%)																							
Vehicle Control							M	D	E	E	M	D	E	E	M	D	E	E					
Vehicle Control							D	E	E	M	D	D	E	E	M	D	E	E					
Vehicle Control								E	E	E	M	D	E	E	E	M	D	E	E				
Vehicle Control										E	M	D	E	E	M	D	E	M	D	D	E		
Vehicle Control										E	M	D	E	M	D	D	E	E	M	D	E		
Vehicle Control								IC	E	E	M	D	E	E	M	D	D	E	E				
Vehicle Control		IC	M	D	E	E	M	D	D	D	D	D											
Vehicle Control						M	D	D	E	E	M	D	D	E	E	M	D						
Vehicle Control										E	M	D	E	E	M	D	E	E	M	D	E		
Vehicle Control								E	E	M	D	D	E	E	M	D	E	E	M				
6.25								E	E	M	D	D	E	E	M	D	E	E	M				
6.25							M	D	E	E	M	D	E	E	M	D	E	E					
6.25										E	M	D	E	E	M	D	E	E	M	D	D		
6.25							M	D	E	E	M	D	E	E	M	D	E	E					
6.25							M	D	E	E	M	D	E	E	M	D	E	E					
6.25									E	M	D	D	E	E	M	D	E	E	E	M			
6.25									E	E	M	D	E	E	M	D	E	E	M	D			
6.25										E	M	D	E	E	M	D	E	E	M	D	E		
6.25							M	D	E	E	M	D	E	E	M	D	E	E					
6.25						E	M	D	D	D	D	D	D	E	M	D	D						
12.5									E	E	M	D	E	E	M	D	E	E	M	D			
12.5										E	D	D	E	E	M	D	E	M	D	D	E		
12.5										E	D	D	E	E	M	D	E	E	M	D	E		
12.5										E	M	D	E	E	M	D	E	E	M	D	E		
12.5								D	E	E	M	D	E	E	M	D	E	E	M				
12.5								D	E	E	M	D	E	E	M	D	E	E	M				
12.5										E	M	D	E	E	M	D	D	E	E	M	D		
12.5							M	D	D	E	E	M	D	E	E	M	D	E					
12.5								M	D	E	E	M	D	E	E	M	D	E	E				
12.5				D	D	D	D	D	D	E	E	M	D	E	E								
25								E	E	M	D	D	E	E	M	D	E	E	M				
25								D	IC	E	M	D	E	E	M	D	E	E	M				
25										E	M	D	E	E	M	D	E	E	M	D	E		
25						M	IC	E	E	M	D	D	E	E	M	D	D						
25										E	M	IC	E	E	E	M	D	E	E	E	M		
25									E	M	D	ND	E	E	M	IC	E	E	M	D			
25										E	M	D	E	E	M	D	E	E	M	D	E		
25						D	D	D	D	E	M	D	E	E	M	D	E						
25										E	M	D	E	E	M	D	E	E	M	D	E		
25						M	D	E	E	M	D	D	E	E	M	D	E						

FIGURE D2
Vaginal Cytology Plots for Female Mice in the 3-Month Dermal Study of Cedarwood Oil
D = diestrus, E = estrus, M = metestrus, IC=insufficient number of cells to determine stage

TABLE D6
Results of Vaginal Cytology Study Using the Transition Matrix Approach for Female Mice in the 3-Month Dermal Study of Cedarwood Oil

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	0.641	
Overall Tests	6.25% vs. Vehicle Controls	0.828	N
Overall Tests	12.5% vs. Vehicle Controls	0.401	N
Overall Tests	25% vs. Vehicle Controls	0.358	
Extended Estrus	Overall	0.848	
Extended Estrus	6.25% vs. Vehicle Controls	0.758	N
Extended Estrus	12.5% vs. Vehicle Controls	0.347	N
Extended Estrus	25% vs. Vehicle Controls	0.994	
Extended Diestrus	Overall	0.985	
Extended Diestrus	6.25% vs. Vehicle Controls	0.882	N
Extended Diestrus	12.5% vs. Vehicle Controls	0.882	N
Extended Diestrus	25% vs. Vehicle Controls	0.777	
Extended Metestrus	Overall	1	
Extended Metestrus	6.25% vs. Vehicle Controls	1	
Extended Metestrus	12.5% vs. Vehicle Controls	1	
Extended Metestrus	25% vs. Vehicle Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	6.25% vs. Vehicle Controls	1	
Extended Proestrus	12.5% vs. Vehicle Controls	1	
Extended Proestrus	25% vs. Vehicle Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	6.25% vs. Vehicle Controls	1	
Skipped Estrus	12.5% vs. Vehicle Controls	1	
Skipped Estrus	25% vs. Vehicle Controls	1	
Skipped Diestrus	Overall	1	
Skipped Diestrus	6.25% vs. Vehicle Controls	1	
Skipped Diestrus	12.5% vs. Vehicle Controls	1	
Skipped Diestrus	25% vs. Vehicle Controls	1	

[a] N means that the dosed group had a lower probability of transitioning to and/or from the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1
Mutagenicity of Cedarwood Oil in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	With 10% rat S9
TA102	0	315 ± 14	405 ± 6
	0.33	278 ± 6	
	1.0	298 ± 3	
	3.3	282 ± 6	421 ± 3
	10	311 ± 17	401 ± 10
	33	240 ± 12	404 ± 9
	100		386 ± 16
	333		346 ± 2
Trial summary		Negative	Negative
Positive control[b]		976 ± 7	1,398 ± 55
TA100	0	160 ± 10	152 ± 14
	0.33	145 ± 3	
	1.0	144 ± 6	
	3.3	141 ± 9	142 ± 2
	10	126 ± 4	136 ± 8
	33	31 ± 11[c]	145 ± 2
	100		124 ± 10
	333		85 ± 7[c]
Trial summary		Negative	Negative
Positive control		630 ± 13	512 ± 86
TA98	0	21 ± 3	34 ± 1
	0.33	17 ± 2	
	1.0	16 ± 2	
	3.3	26 ± 2	31 ± 3
	10	22 ± 4[d]	27 ± 6
	33	14 ± 2[c]	34 ± 3
	100		34 ± 4
	333		0 ± 0[c]
Trial summary		Negative	Negative
Positive control		83 ± 4	676 ± 3

[a] Study was performed at BioReliance Corporation. The detailed protocol is presented by Zeiger *et al*. (1992). Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene.

[c] Slight toxicity

[d] Contamination

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Dermal Administration of Cedarwood Oil for 3 Months[a]

	Concentration (%)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b](%)
Male					
95% Ethanol[d]	0	5	2.00 ± 0.32		3.48 ± 0.09
Cedarwood oil	6.25	5	2.00 ± 0.52	0.5000	3.58 ± 0.14
	12.5	5	2.70 ± 0.20	0.1533	3.46 ± 0.10
	25	5	2.20 ± 0.30	0.3787	3.06 ± 0.15
	50	5	2.20 ± 0.41	0.3787	2.98 ± 0.15
			P=0.209[e]		
Female					
95% Ethanol	0	5	1.60 ± 0.37		3.34 ± 0.19
Cedarwood oil	6.25	5	1.50 ± 0.27	0.5713	3.30 ± 0.11
	12.5	5	1.30 ± 0.41	0.7114	3.50 ± 0.22
	25	5	2.60 ± 0.37	0.0612	3.40 ± 0.16
	50	5	2.40 ± 0.29	0.1027	3.12 ± 0.19
			P=0.011		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). No data are available for 100% mice due to mortality. NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.006
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Cedarwood Oil

Prior to selecting the test article, the NTP investigated the composition of selected components in six lots that were procured as Virginia cedarwood oil from three suppliers. The chromatographic profiles of the six lots were similar to each other. The composition of α-cedrene, β-cedrene, thujopsene, cuparene, and cedrol in these lots ranged from 18% to 40%, 4% to 8%, 16% to 31%, 2% to 4%, and 19% to 26%, respectively. Due to the close similarities between the procured lots, the lot selected for testing was based upon the availability in bulk quantity.

For the current 3-month dermal studies, cedarwood oil was obtained from Texarome, Inc. (Leakey, TX), in one lot (T122303DP). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO) and the study laboratory at Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the cedarwood oil studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a pale yellow oily liquid with a cedar odor, was identified as cedarwood oil by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR spectroscopy. All IR spectra were consistent with the literature spectra for cedarwood oil (Bellanato and Hidalgo, 1971); NMR spectra were consistent with spectra previously obtained from another lot of cedarwood oil. Representative IR and NMR spectra are presented in Figures F1 and F2, respectively.

Physical properties for lot T122303DP were determined by the analytical chemistry laboratory; optical rotation was –26.1°, specific gravity was 0.9581 at 24.8° C, refractive index was 1.5053 at 19° C, and viscosity was 22.6 cP at 22.2° C. The determined values for optical rotation, specific gravity, and refractive index were consistent with the reported values for cedarwood oil (Adams, 1991).

The purity of lot T122303DP was determined by the analytical chemistry laboratory based on a chromatography profile of the major components obtained by using gas chromatography (GC) with flame ionization detection (FID) by system A (Table F1). Five compounds (α-cedrene, β-cedrene, thujopsene, cuparene, and cedrol) were selected as marker compounds and quantitated using standards obtained from Sigma-Aldrich Corporation (St. Louis, MO). Weight percent concentrations of the five selected marker compounds were determined to be α-cedrene, 27.01%, β-cedrene, 6.05%, thujopsene, 19.64%, cuparene, 0.96%, and cedrol, 21.83%; these results were not significantly changed by homogenization of the bulk chemical (heating to 50° C) prior to analysis.

The analytical chemistry laboratory also determined the components of lot T122303DP using GC with mass spectrometry (MS) detection by system B to profile the major components of the test article. Ten marker compounds having peak areas of at least 1% of the total peak area were identified in this analysis; α-cedrene, β-cedrene, thujopsene, β-chamigrene, α-alaskene, α-cuprenene, β-himachalene, cuparene, cedrol, and widdrol. Headspace analysis for volatiles were conducted on samples of this lot using GC/MS by system B both before and after homogenization at 50° C, and 15 components were identified in the chromatographic profiles: limonene (or an isomer), iso-italicene (or an isomer), italicene (or an isomer), α-cedrene, *cis*-β-farnesene (or an isomer), β-selinene (or an isomer), β-cedrene, thujopsene, β-chamigrene (or an isomer), α-alaskene (or an isomer), α-cuprenene (or an isomer), β-himachalene (or an isomer), cuparene, cedrol, and widdrol. No significant differences were observed between samples analyzed before and after homogenization. Taken together, the results of these characterization assays indicated that the composition of lot T122303DP was consistent with that reported in the literature for cedarwood oil (Adams, 1989, 1991, 2001).

One aliquot of cedarwood oil was submitted to Covance Laboratories, Inc. (Madison, WI) for nutritional and contaminant testing using standard methods. For this lot, mercury and molybdenum were detected at 13.2 ppb and 18.2 ppb, respectively. The other metals (antimony, arsenic, cadmium, and lead) were below the limit of quantitation of the assay (<10 ppb). For mycotoxin analyses, levels of aflatoxins B1, B2, G1, and G2 were all less than 0.500 ppb; levels of ochratoxin and zearalinine were less than 1 and 20 ppb, respectively. The pesticide screen experienced significant matrix effects that prevented quantitation of analytes.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory. GC/FID was performed using system A for samples stored at –20°, 5°, 25°, or 60° C in sealed amber glass vials; additional freeze/thaw analyses were also performed every 2 to 3 days during the 2-week stability studies. Stability was confirmed for at least 2 weeks for samples stored at temperatures up to 25° C in sealed amber glass vials. Freeze/thaw analyses indicated no decomposition due to repeated freezing and thawing.

To ensure stability, the bulk chemical was stored at approximately 25° C in the original sealed amber glass shipping bottles. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month studies using GC/FID by system A; no degradation of the bulk chemical was detected.

Ethanol

USP grade 95% ethanol was obtained from Spectrum Chemicals & Laboratory Products (Gardena, CA) in one lot (TP0179) and from AAPER Alcohol (Shelbyville, KY) in one lot (02K2JWB). Lot TP0179 was used in the 3-month dermal studies, and lot 02K2JWB was used in the dose formulation stability studies.

Lot TP0179, a clear liquid, was identified as ethanol by the study laboratory using IR spectroscopy; the IR spectrum was consistent with a literature spectrum (*Aldrich*, 1981) of ethanol. The purity of lot TP0179 was determined by the study laboratory using GC/FID by system C; no impurities greater than 0.1% of the total peak area were detected. No benzene was detected in the test article using GC/FID by system D.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared four times by mixing cedarwood oil and 95% ethanol to give the required concentrations (Table F2). The dose formulations were stored at approximately 25° C in amber glass bottles sealed with Teflon®-lined lids for up to 34 days.

Stability studies of a 9.5 mg/mL (0.95%) formulation in 95% ethanol were performed by the analytical chemistry laboratory using GC/FID by system E (Table F1). Based on these studies, it was determined that cedarwood oil formulations could be stored in amber glass containers sealed with Teflon®-lined lids for 1 day with expected losses of 6.2% or less for all components or stored for 42 days with expected losses of 10.4% or less. Cedarwood oil formulations were stable for up to 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of cedarwood oil were conducted by the study laboratory using GC/FID by a system similar to system E. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations analyzed for rats and all 14 dose formulations for mice were within 10% of the target concentrations (Table F3). Animal room samples of these dose formulations were also analyzed; all 15 for rats and all 14 for mice were within 10% of the target concentrations.

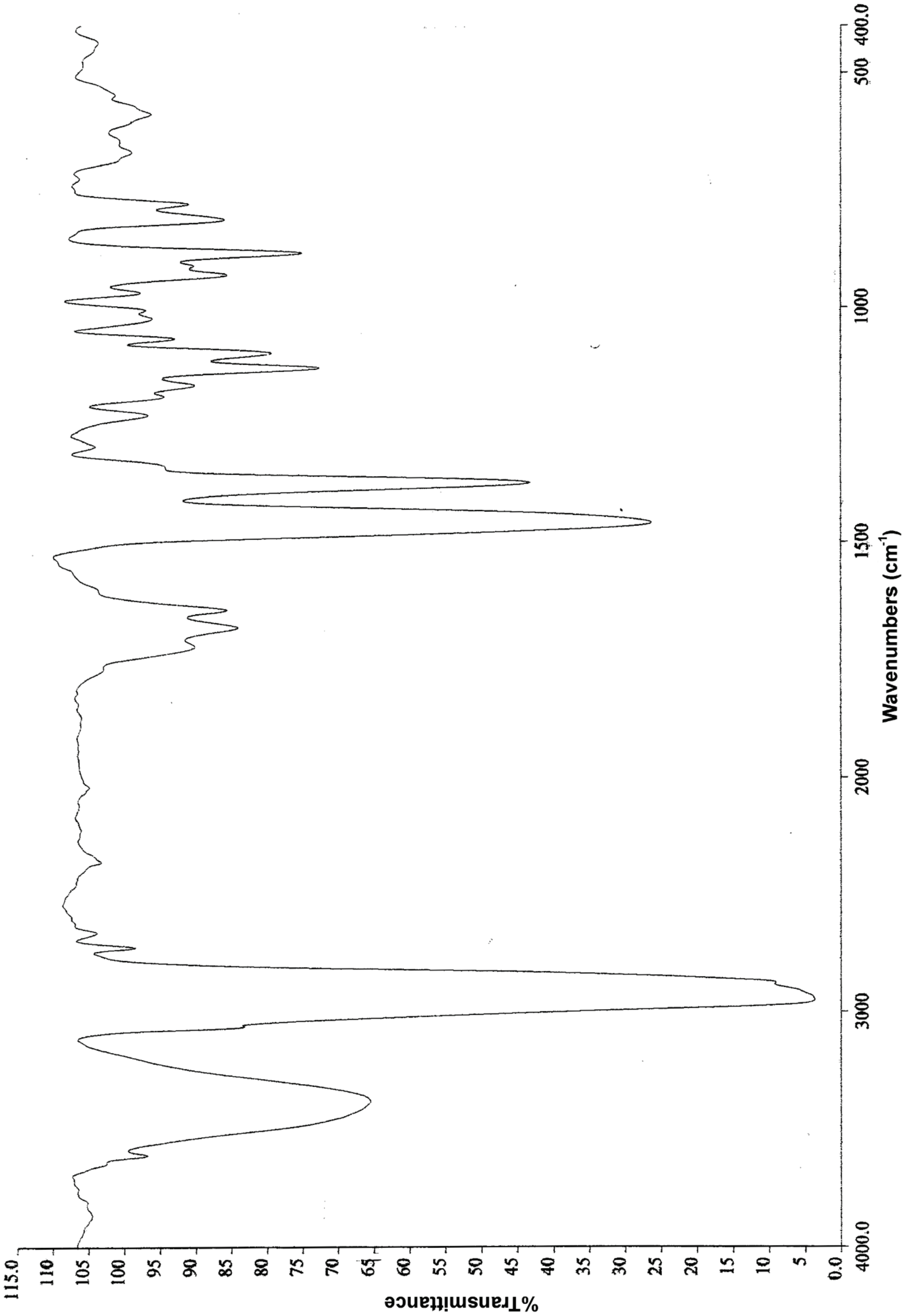


FIGURE F1
Infrared Absorption Spectrum of Cedarwood Oil

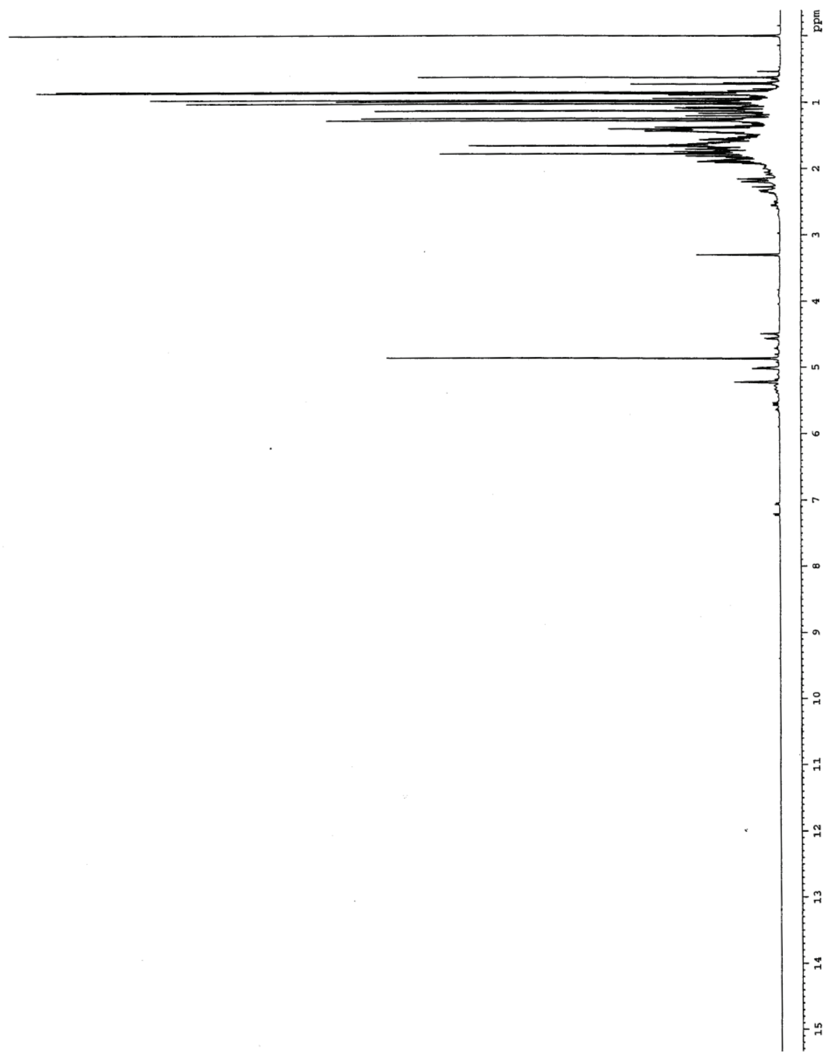


FIGURE F2
Proton Nuclear Magnetic Resonance Spectrum of Cedarwood Oil

TABLE F1
Gas Chromatography Systems Used in the 3-Month Dermal Studies of Cedarwood Oil[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-WAX, 30 m × 0.53 mm, 1.0 µm film (J&W Scientific, Folsom, CA)	Helium at 10 mL/minute	60° C for 2 minutes, then 3° C/minute to 220° C
System B			
Mass spectrometry	DB-WAX, 30 m × 0.25 mm, 0.5 µm film (J&W Scientific)	Helium at 1 mL/minute	60° C for 3 minutes, then 3° C/minute to 220° C
System C			
Flame ionization	DB-WAX, 30 m × 0.53 mm, 1.0 µm film (J&W Scientific)	Helium at 10 mL/minute	40° C for 5 minutes, then 10° C/minute to 220° C, held for 5 minutes
System D			
Flame ionization	Rtx®-5, 30 m × 0.53 mm, 1.5 µm film (Restek, Bellefonte, PA)	Helium at 10 mL/minute	40° C for 3 minutes, then 10° C/minute to 200° C, held for 3 minutes
System E			
Flame ionization	DB-WAX, 30 m × 0.53 mm, 1.0 µm film (J&W Scientific)	Helium at 10 mL/minute	60° C for 3 minutes, then 3° C/minute to 220° C, held for 2 minutes

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Santa Clara, CA; systems A, C, D, and E) or Thermo Fisher Scientific, Inc., (Waltham, MA; system B). The mass spectrometer was manufactured by Fisons (Sanofi, Bridgewater, NJ).

TABLE F2
Preparation and Storage of Dose Formulations in the 3-Month Dermal Studies of Cedarwood Oil

Preparation
For the 6.25%, 12.5%, 25%, and 50 % formulations, the appropriate amount of cedarwood oil was measured in a graduated cylinder and transferred with at least three ethanol rinses into a calibrated mixing container that contained 95% ethanol, diluted to a final volume with 95% ethanol, shaken by hand for 2 minutes, inverted at least 10 times, and then stirred on a stirplate for approximately 5 minutes at a speed that produced a vigorous vortex. Undiluted cedarwood oil was dispensed directly into amber glass dose formulation bottles as the 100% (neat) formulation. The dose formulations were prepared four times during the studies.

Chemical Lot Number
T122303DP

Maximum Storage Time
34 days

Storage Conditions
Stored in amber glass bottles sealed with Teflon®-lined lids at approximately 25° C.

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies of Cedarwood Oil[a]

Date Prepared	Date Analyzed	Target Concentration (%)	Determined Concentration[b] (%)	Difference from Target (%)
Rats				
May 31, 2005	June 3-4, 2005	6.25	6.244	0
		12.5	12.31	–2
		25	25.20	+1
		50	53.20	+6
		100	101.8	+2
	July 8-10, 2005[c]	6.25	6.291	+1
		12.5	12.65	+1
		25	25.75	+3
		50	52.15	+4
	July 11-12, 2005[c]	100	97.87	–2
June 23, 2005	June 24-25, 2005	6.25	6.071	–3
		12.5	12.20	–2
		25	24.69	–1
		50	50.11	0
		100	99.63	0
	July 27-29, 2005[c]	6.25	6.213	–1
		12.5	12.25	–2
		25	25.01	0
		50	49.53	–1
		100	97.89	–2
August 16, 2005	August 16-17, 2005	6.25	6.209	–1
		12.5	11.97	–4
		25	23.68	–5
		50	49.21	–2
		100	93.85	–6
	September 19-22, 2005[c]	6.25	6.380	+2
		12.5	12.79	+2
		25	25.46	+2
		50	52.34	+5
		100	101.2	+1
Mice				
May 31, 2005	June 3-4, 2005	6.25	6.244	0
		12.5	12.31	–2
		25	25.20	+1
		50	53.20	+6
		100	101.8	+2
	July 8-10, 2005[d]	6.25	6.256	0
		12.5	12.59	+1
		25	25.64	+3
		50	53.00	+6
	July 11-12, 2005[d]	100	98.54	–1

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies of Cedarwood Oil

Date Prepared	Date Analyzed	Target Concentration (%)	Determined Concentration (%)	Difference from Target (%)
Mice (continued)				
June 23, 2005	June 24-25, 2005	6.25	6.071	–3
		12.5	12.20	–2
		25	24.69	–1
		50	50.11	0
		100	99.63	0
	July 27-29, 2005[d]	6.25	5.929	–5
		12.5	11.84	–5
		25	23.87	–5
		50	47.41	–5
		100	93.06	–7
August 16, 2005	August 16-17, 2005	6.25	6.209	–1
		12.5	11.97	–4
		25	23.68	–5
		50	49.21	–2
	September 19-22, 2005[d]	6.25	6.377	+2
		12.5	12.82	+3
		25	25.45	+2
		50	52.20	+4

[a] The vehicle control was also analyzed and was below the limit of quantitation.
[b] Results of duplicate analyses.
[c] Animal room samples for rats
[d] Animal room samples for mice

APPENDIX G
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE G1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE G2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE G3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.8 ± 0.44	14.4 – 15.3	4
Crude fat (% by weight)	8.1 ± 0.17	7.9 – 8.3	4
Crude fiber (% by weight)	9.3 ± 0.46	8.7 – 9.8	4
Ash (% by weight)	4.8 ± 0.16	4.6 – 5.0	4
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.070	0.670 – 0.970	22
Cystine	0.220 ± 0.024	0.150 – 0.250	22
Glycine	0.701 ± 0.041	0.620 – 0.800	22
Histidine	0.352 ± 0.077	0.270 – 0.680	22
Isoleucine	0.546 ± 0.044	0.430 – 0.660	22
Leucine	1.095 ± 0.067	0.960 – 1.240	22
Lysine	0.711 ± 0.114	0.310 – 0.860	22
Methionine	0.409 ± 0.046	0.260 – 0.490	22
Phenylalanine	0.628 ± 0.040	0.540 – 0.720	22
Threonine	0.505 ± 0.043	0.430 – 0.610	22
Tryptophan	0.150 ± 0.028	0.110 – 0.200	22
Tyrosine	0.401 ± 0.061	0.280 – 0.540	22
Valine	0.665 ± 0.043	0.550 – 0.730	22
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.259	3.49 – 4.55	22
Linolenic	0.30 ± 0.032	0.21 – 0.35	22
Vitamins			
Vitamin A (IU/kg)	4,373 ± 57	3,710 – 5,080	4
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	80.6 ± 22.03	27.0 – 124.0	22
Thiamine (ppm)[b]	8.0 ± 1.06	6.6 – 9.0	4
Riboflavin (ppm)	7.6 ± 2.89	4.20 – 17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4 – 98.2	22
Pantothenic acid (ppm)	26.9 ± 12.63	17.4 – 81.0	22
Pyridoxine (ppm)[b]	9.54 ± 1.99	6.44 – 13.7	22
Folic acid (ppm)	1.62 ± 0.48	1.15 – 3.27	22
Biotin (ppm)	0.32 ± 0.10	0.20 – 0.704	22
Vitamin B_{12} (ppb)	53.6 ± 39.6	18.3 – 174.0	22
Choline (ppm)[b]	2,846 ± 485	1,820 – 3,790	22
Minerals			
Calcium (%)	0.964 ± 0.054	0.903 – 1.030	4
Phosphorus (%)	0.558 ± 0.019	0.535 – 0.576	4
Potassium (%)	0.666 ± 0.030	0.626 – 0.733	22
Chloride (%)	0.386 ± 0.039	0.300 – 0.474	22
Sodium (%)	0.189 ± 0.016	0.160 – 0.222	22
Magnesium (%)	0.216 ± 0.062	0.185 – 0.490	22
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	186 ± 39.2	135 – 311	22
Manganese (ppm)	51.4 ± 10.28	21.0 – 73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3 – 78.5	22
Copper (ppm)	7.01 ± 2.562	3.21 – 16.3	22
Iodine (ppm)	0.503 ± 0.206	0.158 – 0.972	22
Chromium (ppm)	0.694 ± 0.276	0.330 – 1.380	22
Cobalt (ppm)	0.256 ± 0.164	0.098 – 0.864	22

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE G4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 ± 0.040	0.19 – 0.28	4
Cadmium (ppm)	0.05 ± 0.004	0.04 – 0.05	4
Lead (ppm)	0.10 ± 0.019	0.08 – 0.12	4
Mercury (ppm)	<0.02		4
Selenium (ppm)	0.26 ± 0.007	0.25 – 0.26	4
Aflatoxins (ppb)	<5.00		4
Nitrate nitrogen (ppm)[c]	8.58 ± 0.687	7.9 – 9.4	4
Nitrite nitrogen (ppm)[c]	4.6 ± 1.27	2.81 – 5.81	4
BHA (ppm)[d]	<1.0		4
BHT (ppm)[d]	<1.0		4
Aerobic plate count (CFU/g)	10 ± 0.0	10	4
Coliform (MPN/g)	3.0 ± 0.0	3.0	4
Escherichia coli (MPN/g)	<10		4
Salmonella (MPN/g)	Negative		4
Total nitrosoamines (ppb)[e]	4.2 ± 1.35	3.2 – 6.1	4
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 1.11	1.4 – 3.9	4
N-Nitrosopyrrolidine (ppb)[e]	1.7 ± 0.39	1.3 – 2.2	4
Pesticides (ppm)			
α-BHC	<0.01		4
β-BHC	<0.02		4
γ-BHC	<0.01		4
δ-BHC	<0.01		4
Heptachlor	<0.01		4
Aldrin	<0.01		4
Heptachlor epoxide	<0.01		4
DDE	<0.01		4
DDD	<0.01		4
DDT	<0.01		4
HCB	<0.01		4
Mirex	<0.01		4
Methoxychlor	<0.05		4
Dieldrin	<0.01		4
Endrin	<0.01		4
Telodrin	<0.01		4
Chlordane	<0.05		4
Toxaphene	<0.10		4
Estimated PCBs	<0.20		4
Ronnel	<0.01		4
Ethion	<0.02		4
Trithion	<0.05		4
Diazinon	<0.10		4
Methyl chlorpyrifos	0.091 ± 0.066	0.025 – 0.166	4
Methyl parathion	<0.02		4
Ethyl parathion	<0.02		4
Malathion	0.358 ± 0.118	0.233 – 0.500	4
Endosulfan I	<0.01		4
Endosulfan II	<0.01		4
Endosulfan sulfate	<0.03		4

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX H
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected, allowed to clot, and the serum was separated. The serum samples were processed appropriately, sent to BioReliance Corporation (Rockville, MD), and evaluated for the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
ELISA	
PVM (pneumonia virus of mice)	Study start, 4 weeks, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study start, 4 weeks, study termination
Sendai	Study start, 4 weeks, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 4 weeks, study termination
MICE	
ELISA	
Ectromelia virus	Study start, 4 weeks, study termination
EDIM (epizootic diarrhea of infant mice)	Study start, 4 weeks, study termination
GDVII (mouse encephalomyelitis virus)	Study start, 4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	Study start, 4 weeks, study termination
Mouse adenoma virus-1	Study start, 4 weeks, study termination
MHV (mouse hepatitis virus)	Study start, 4 weeks, study termination
MMV VP2 (mouse minute virus)	Study start, 4 weeks, study termination
MPV VP2 (mouse parvovirus)	Study start, 4 weeks, study termination
PVM	Study start, 4 weeks, study termination
Reovirus	Study start, 4 weeks, study termination
Sendai	Study start, 4 weeks, study termination

RESULTS

All test results were negative.